

ANTI-INFLAMMATORY POTENTIAL OF DIFFERENT EXTRACTS ISOLATED FROM THE ROOTS OF *FICUS LACOR* BUCH. HUM AND *MURRAYA KOENIGII* L. SPRENG

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Abstract - The present study was undertaken to evaluate the anti-inflammatory potential of *Murraya koenigii* root extracts petroleum ether, ethyl acetate and chloroform (MKPE, MKEA and MKCF, respectively) and *Ficus lacor* aerial root extracts petroleum ether, ethyl acetate, chloroform and ethanol (FLPE, FLET, FLCF and FLET, respectively) at doses of 50 and 100 mg/kg body weight (b.w.) using animal models of acute inflammation (carrageenan-, histamine- and serotonin-induced inflammation). The results of the *Murraya koenigii* roots chloroform extract caused 66.4% inhibition and the ethanol extract of *Ficus lacor* aerial roots caused 68.3% inhibition at the dose of 50 mg/kg b.w. At a higher dose of 100 mg/kg b.w., MKPE and MKCF showed 55.10% and 70.10% inhibition, respectively. FLPE and FLET showed 74.50% and 75.40% inhibition, respectively, in the carrageenan-induced inflammation model. In histamine-induced inflammation, the MKCF showed 60% inhibition, and 67.01% and 68.02% inhibition with the petroleum ether and ethanol extracts, respectively, in *Ficus lacor* aerial roots at the dose of 50 mg/kg b.w. At a higher dose (100 mg/kg b.w.), MKCF showed 64% inhibition. FLPE and FLET showed 70.13% and 74.01% inhibition, respectively; 62.15% and 66.10% inhibition was observed with the petroleum ether and ethanol extracts of *Ficus lacor* aerial roots at 50 mg/kg b.w. At higher dose (100 mg/kg b.w.), FLPE and FLET showed 69.10% and 68.72% inhibition in serotonin-induced inflammation.

Key words: Anti-inflammatory; *Murraya koenigii*; *Ficus lacor*; roots.

INTRODUCTION

Inflammation is a complex biological process involving several chemical mediators that are induced by the vascular tissue of the body when it comes in contact with harmful stimuli such as pollens, irritants, pathogens and damaged cells. The process of inflammation involves several events and mediators that are potent chemical substances found in the body tissues, such as prostaglandins, leukotrienes, prostacyclins, lymphokines, and chemokines such as interferon- α (IFN- α), γ , interleukin (IL)-1, IL-8, histamine, 5-hydroxytryptamine (5-HT), and tissue necrosis factor- α (Serhan and Savill, 2005).

Anti-inflammatory drugs of synthetic origin are classified as steroidal and nonsteroidal anti-inflammatory agents. The origin of these chemical compounds started when salicylates were isolated from the leaf extract of willow bark (*Salix alba*) and were used by the people of North America in 200 BC; they are regarded as the first generation of anti-inflammatory agents (Rainsford and Whitehouse 1980). These were followed by the discovery of second- and third-generation compounds with preferential and selective cyclooxygenase (COX2) inhibitory activities, such as nimesulide, nabumetone, celecoxib, rofecoxib, valdecoxib and etoricoxib. Apart from the nonsteroidal drugs, various corticosteroids such as

hydrocortisone, betamethasone, and beclomethasone are primarily used as anti-inflammatory agents (MacLennan et al., 1996; Ang-Lee et al. 2001).

Some common side effects of these synthetic drugs include gastric irritation, ulceration, bleeding, renal failure, interstitial nephritis, hepatic failure, headache, thrombocytopenia, hemolytic anemia, asthma exacerbation, skin rashes, angioedema and pruritis (Anonymous, 1993; Anonymous, 1996). Hence, the approach for treating inflammatory diseases by herbal drugs has the keen interest of researchers. From a global study, it has been seen that the market for herbal drugs in the treatment of inflammatory diseases constitutes 83% worldwide and is expected to reach a value of over 95% in the forthcoming years due to the increased acceptability of these preparations (Bent and Ko 2004; Boulata and Nace, 2000). According to the WHO, about 80% of the world's population relies on traditional drugs to treat various types of ailments (Sindhu et al., 2010). *Murraya koenigii* Linn (Rutaceae), commonly known as Meethi neem, is an aromatic, deciduous shrub, found throughout India (Pande et al., 2009). In traditional medicine, it is used as an antiemetic, antidiarrheal, anti-dysentery, febrifuge, blood purifier, tonic, stomachic, flavoring agent in curries and chutneys (Prajapati et al., 2003). The plant contains various types of phytochemicals such as alkaloids, flavonoids, amino acids and saponins (Sindhu and Arora, 2012). The reported pharmacological activities are anti-oxidative, cytotoxic (Shah and Juvekar, 2006), antimicrobial (Manfred et al., 1985), anti-diabetic and cholesterol-reducing (Kesari et al., 2005) and antiulcer (Xie et al., 2006), with positive inotropic effect (Rahman and Gray, 2005). *Ficus lacor* Buch. -Ham is a synonym of *Ficus infectoria* Roxb. It is locally known as pilkhan, and it is a large deciduous, rapidly growing closely foliaceous tree about 20 m in height with a fine shaped crown. It is widely distributed in tropical and subtropical regions of the world. It also grown in various humid regions in India (Chopra et al., 1956). The bark of the plant in the traditional medicine of India is used for the treatment of ulcers, for expelling roundworms and for the treatment of leucorrhoea. The leaves are also used for

treating various skin problems (Gamble, 1922; Nadkarni and Nadkarni, 1976). Phytochemical screening of the plant revealed the presence of terpenoids, sterol, amino acids and flavonoids, etc. Medicinal plants or isolated bioactive constituents form one of the major sources of raw materials for drugs (Balgia, 2003) in preventive or curative applications (Jain and Yadav, 1994). The present study was undertaken to evaluate the anti-inflammatory potential of *Murraya koenigii* root extracts and aerial root extracts of *Ficus lacor*.

MATERIALS AND METHODS

Murraya koenigii roots were collected Jhansla village, Patiala (Punjab) and aerial roots of *Ficus lacor* were collected during the month of the July 2009 from Panchkula (Haryana), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division, with ref. no. NISCAIR/RHMD/Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of Pharmacy, Chitkara University, Punjab.

Preparation of extracts

The powdered material was subjected to successive hot extraction (Soxhlet) with different solvents in increasing order of polarity from petroleum ether (PE), ethyl acetate (EA), chloroform (CF) and ethanol (ET), followed by distillation and concentration in a water bath (Mukherjee, 2003).

Phytochemical screening

The phytochemical screening of different extracts using various chemical and reagents was carried out following the method of Harbone (1973)

Experimental animals

Wistar rats of either sex (weighing 160-200 g) were used as per experimental protocols (IAEC/CCP/12/

PR-005) after approval from the Institutional Animal Ethical Committee, Chitkara College of Pharmacy, Chitkara University, Jhansla, Rajpura. The animals were housed under standard environmental conditions ($25\pm 2^{\circ}\text{C}$ and relative humidity $50\pm 5\%$) and fed with standard diet and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. Each group comprised 6 rats.

Toxicity studies

Acute toxicity study was performed for ethanol extract of both plants according to the acute toxic classic method as per OECD guidelines (Anonymous, 2000). Albino rats were used for acute toxicity study. The animals were fasted overnight providing only water, after which the extract was administered orally at the dose of 100 mg/kg. The rats were observed for 14 days. If mortality occurred in two out of three animals, then the dose administered was considered toxic. If mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses – 50, 200 and 2 000 mg/kg b.w. The animals were observed for toxic symptoms such as behavioral changes, changes in locomotor functions, convulsions and mortality for 72 h.

Dose

Indomethacin (2.5mg/kg b.w.) and Pyrilamine (1 mg/kg b.w.) were selected as a standard for anti-inflammatory activity. The doses of different extracts were 50 and 100 mg/kg b.w. The stock solution of standard was prepared in DMSO.

PHARMACOLOGICAL SCREENING

Inhibition of carrageenan-induced paw edema in rats

Control group I was given normal saline 1 h before the carrageenan injection. Experimental groups were given doses of various extracts or fractions in 0.5ml of normal saline, injected intraperitoneally and orally 1 h before injection of 0.1 ml of 1% carrageenan solu-

tion in the right hind paw under the plantar aponeurosis (s.c) for induction of edema. The volume of paw edema was determined by plethysmometer, a dose response relationship was established for both oral and i.p. dose and a correlation was established between i.p. and oral doses producing maximum anti-inflammatory effect. The reference group was given Indomethacin 2.5 mg/kg 1h before the carrageenan injection. Percentage inhibition of edema relative to the control group was calculated as described by Winter et al. (1962)

Inhibition of histamine and serotonin induced paw edema in rats

In another set of experiments, serotonin and histamine (0.1 ml of 1mg/ml of both) were used as phlogistic agents. The various extracts, standard pyrilamine and control vehicle (solution of 2.5% DMSO and 2.5% Tween 20) were administered intraperitoneally 1 h before the injection of the inflammatory mediators in their respective groups. Different doses of extract or fractions were injected intraperitoneally with vehicle to establish the dose response relationship; 0.1ml serotonin (1mg/ml) or histamine (1mg/ml) was injected and response noted at 30 min and 60 min, respectively. Pyrilamine maleate (1 mg/kg) was used as the antagonist (reference) of histamine and as a standard drug in the reference group. The volume of paw edema was determined by plethysmometer (Singh et al., 1996).

Statistical analysis

Data obtained from animal experiments were expressed as mean standard error (\pm S.E.M.). Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keuls post hoc tests. Significance of data was expressed as * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of various extracts of *Ficus lacor* plants revealed the presence of phytoconstituents such as glycosides,

Table 1. Inhibition of Carragenan induced edema (paw volume) in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Groups	Isolated Fractions	Dose mg/kg i.p.	M.E.V and SEM	P.I. (% Inhibition)
I	Normal	(Normal saline) Arthritic control	0.37±0.02	--
II.	MKPE	50	0.26± 0.056	32.43
III.	MKEA	50	0.24± 0.049*	36.8
IV.	MKCF	50	0.14± 0.051 ***	66.4
V.	FLPE	50	0.21± 0.058 **	41.9
VI.	FLEA	50	0.26±0.048	30.7
VII.	FLCF	50	0.25± 0.058*	35.9
VIII.	FLET	50	0.12±0.049 ***	68.3
IX.	Indomethacin	2.5 mg	0.09±0.057 ***	81.6

Table 2. Inhibition of Carragenan induced edema (paw volume) in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Groups	Isolated Fractions	Dose mg/kg i.p.	M.E.V and SEM	P.I. (% Inhibition)
I	Normal	(Normal saline) Arthritic control	0.37±0.02	--
II.	MKPE	100	0.20±0.058 **	55.10
III.	MKEA	100	0.22± 0.050 *	38.80
IV.	MKCF	100	0.10±0.050 ***	70.10
V.	FLPE	100	0.09±0.040 ***	74.50
VI.	FLEA	100	0.26±0.048	30.70
VII.	FLCF	100	0.21± 0.058 *	41.90
VIII.	FLET	100	0.10±0.037 ***	75.40
IX.	Indomethacin	2.5 mg	0.08±0.057	80.80

MEV Values represent Mean± SEM; MEV = Mean Edema volume & PI = Percentage inhibition; Group I- Edema Control (saline); Group II- Arthritic rats treated with from MKPE fraction, Group III- Arthritic rats treated with MKEA fraction, Group IV- Arthritic rats treated MKCF fraction from *M. koenigii*, Group V - Arthritic rats treated with FLPE fraction, Group VI- Arthritic rats treated with FLEA fraction, Group VII- Arthritic rats treated with FLCF fraction, Group VIII- Arthritic rats treated with FLET fraction from *Ficus lacor*, Group IX- Arthritic rats treated with Indomethacin 2.5mg/kg. *p<0.05, **p<0.01, *** p <0.001 as compared to arthritic control.

alkaloids, phenolic and tannins, flavonoids, and *Murraya koenigii* showed the presence of alkaloids, flavonoids, carbohydrates, sterol and amino acids in various extracts. The ethanol extract of both plants did not produce any toxic effects or symptoms in rats. Therefore, these extracts were considered safe for

further pharmacological investigation. The anti-inflammatory potential of the various extracts of *Ficus lacor* aerial roots and *Murraya koenigii* roots against acute pedal edema is shown in Tables 1-6, and show significant anti-inflammatory activity comparable to standard drugs.

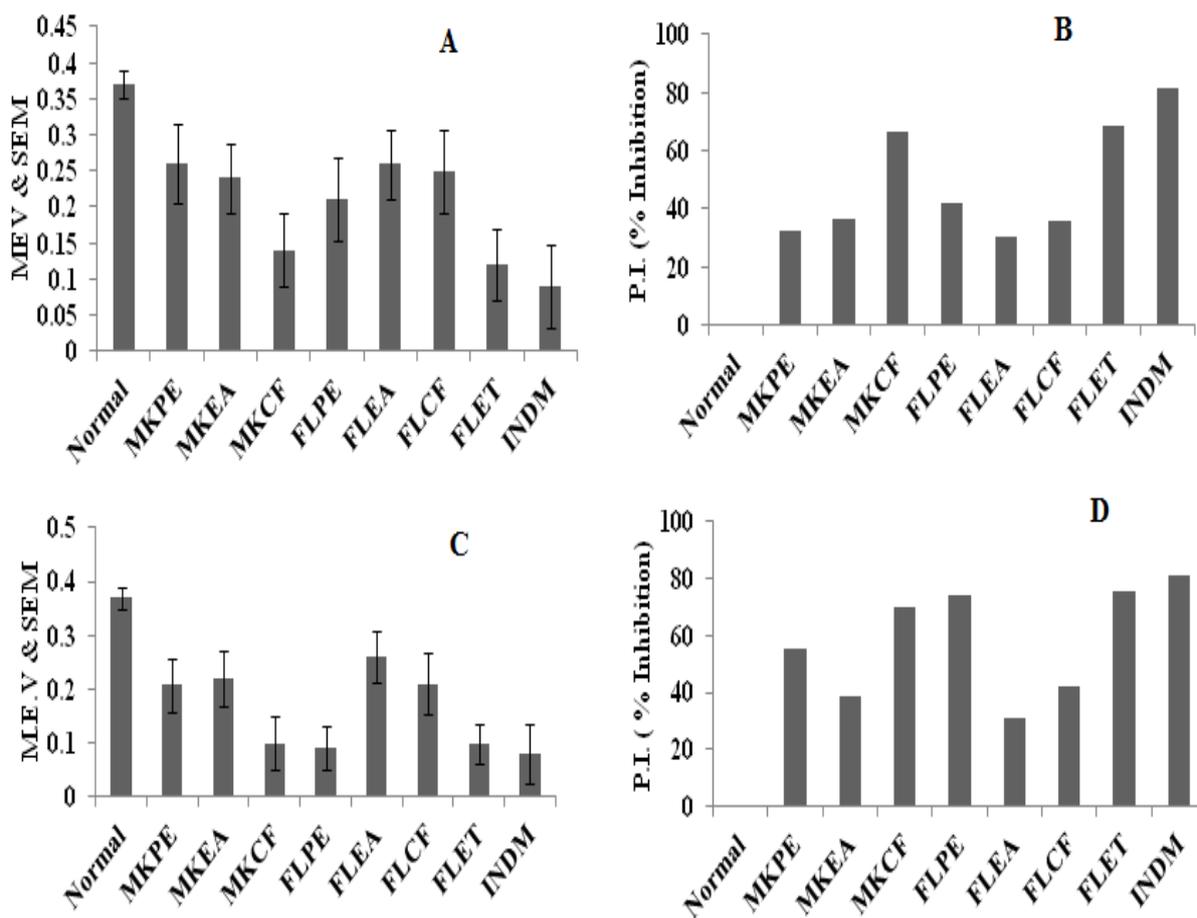


Fig. 1 Inhibition of Carrageenan induced edema (paw volume) in rats by different extracts from *Murraya koenigii* and *Ficus lacor*, A and B Shows at lower dose and C and D at higher doses.

Carrageenan-induced paw edema in rats

The *Murraya koenigii* roots chloroform extract showed 66.4% inhibition and 68.3% inhibition was shown in the ethanol extract of *Ficus lacor* aerial roots at the dose of 50mg/kg b.w. as shown in Table 1 and figure 1(A and B). At higher dose (100 mg/kg b.w.), *Murraya koenigii* root extracts MKPE and MKCF showed 55.10% and 70.10% inhibition, respectively. *Ficus lacor* extracts FLPE and FLET showed 74.50% and 75.40% inhibition, respectively, as shown in Table 2 and figure 1 (C and D).

Histamine-induced paw edema in rats

The *Murraya koenigii* roots' chloroform extract showed 60% inhibition, and 67.01% 68.02% inhibition was shown in the petroleum ether and ethanol extract of *Ficus lacor* aerial roots, respectively, at the dose of 50mg/kg b.w. as shown in Table 3 and figure 2 (A and B). At higher dose (100 mg/kg b.w.), *Murraya koenigii* roots' extracts MKCF showed 64% inhibition. *Ficus lacor* extracts FLPE and FLET showed 70.13% and 74.01% inhibition, respectively, as shown in Table 4 and figure 2 (C and D).

Table 3. Inhibition of histamine induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Histamine	
			MEV	PI
I.	Normal	(Normal saline) Arthritic control	0.37±0.02	--
II.	MKPE	50	0.42±0.04	13.66
III.	MKEA	50	0.27±0.10	24.10
IV.	MKCF	50	0.15±0.06**	60.00
V.	FLPE	50.	0.10±0.04***	67.01
VI.	FLEA	50	0.22±0.04 **	43.46
VII.	FLCF	50	0.24±0.01	38.66
VIII.	FLET	50.	0.12±0.04***	68.02
IX	Pyrilamine	1mg/kg	0.08±0.04**	79.01

Table 4. Inhibition of histamine induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Histamine	
			MEV	PI
I.	Normal	(Normal saline) Arthritic control	0.37±0.02	--
II.	MKPE	100	0.23±0.04*	25.1
III.	MKEA	100	0.22±0.10	35.66
IV.	MKCF	100	0.13±0.04**	64.00
V.	FLPE	100	0.10±0.04***	70.13
VI.	FLEA	100	0.21±0.04 **	41.66
VII.	FLCF	100	0.25±0.01	38.66
VIII.	FLET	100.	0.9±0.04***	74.01
IX	Pyrilamine	1mg/kg	0.06±0.04**	82.01

MEV Values represent Mean± SEM; MEV = Mean Edema volume & PI = Percentage inhibition; Group I- Edema Control (saline); Group II- Arthritic rats treated with MKPE fraction, Group III- Arthritic rats treated with MKEA fraction, Group IV- Arthritic rats treated MKCF fraction from *M. koenigii*, Group V - Arthritic rats treated with FLPE fraction, Group VI- Arthritic rats treated with FLEA fraction, Group VII- Arthritic rats treated with FLCF fraction, Group VIII- Arthritic rats treated with FLET fraction from *Ficus lacor*, Group IX- Arthritic rats treated with Pyrilamine 1mg/kg. *p<0.05, **p<0.01, *** p <0.001 as compared to arthritic control

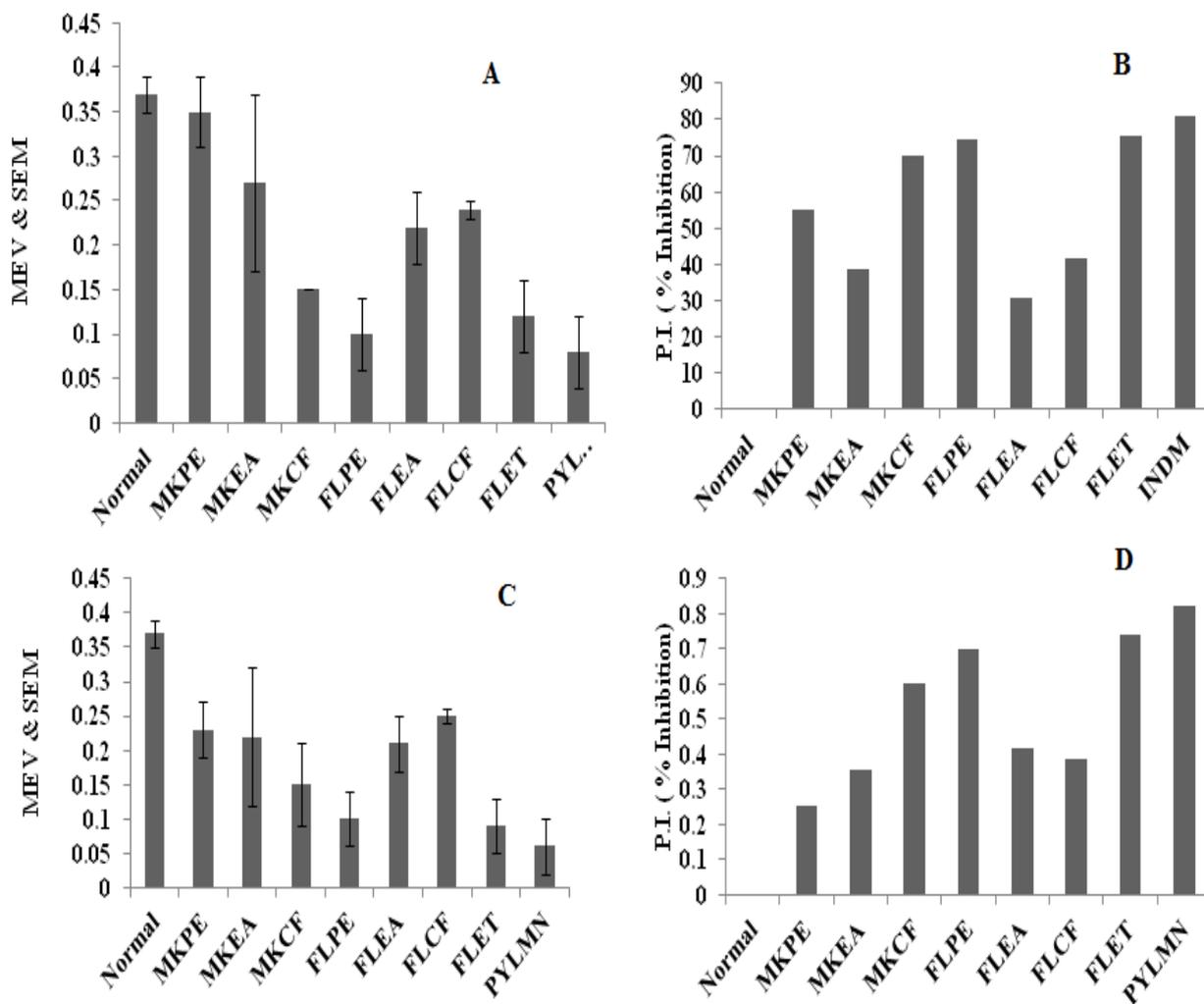


Fig. 2 Inhibition of histamine induced paw edema in rats by different extracts from *Murraya koenigii* and *Ficus lacor*, A and B Shows at lower dose and C and D at higher doses.

Serotonin-induced paw edema in rats

The 62.15% and 66.10% inhibition in petroleum ether and ethanol extracts of *Ficus lacor* aerial roots at the dose of 50mg/kg b.w. are shown in Table 5 and figure 3 (A and B). At higher dose (100 mg/kg b.w.), *Ficus lacor* extracts FLPE and FLET showed 69.10 and 68.72% inhibition, respectively, as shown in Table 6 and figure 3 (C and D).

From these results, it can be concluded that MKPE, MKCF, FLPE and FLET at the dose of 100 mg/kg b.w. showed significant anti-inflammatory activity due to presence of phytoconstituents.

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Table 5. Inhibition of Serotonin induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Serotonin	
			MEV	PI
I.	Normal	(Normal saline) Arthritic control	0.53±0.04	--
II.	MKPE	50	0.46±0.02	14.14
III.	MKEA	50	0.42±0.02*	22.64
IV.	MKCF	50	0.38±0.02*	31.64
V.	FLPE	50.	0.20±0.01***	62.15
VI.	FLEA	50	0.41±0.02 *	22.64
VII.	FLCF	50	0.39±0.07*	28.64
VIII.	FLET	50.	0.13±0.01**	66.10
IX	Pyrilamine	1mg/kg	0.12±0.01***	80.10

Table 6: Inhibition of Serotonin induced paw edema in rats by various fractions from *Murraya koenigii* and *Ficus lacor*

Group	Isolated Fractions	Dose mg/kg	Serotonin	
			MEV	PI
I.	Normal	(Normal saline) Arthritic control	0.53±0.04	--
II.	MKPE	100	0.43±0.02	18.14
III.	MKEA	100	0.40±0.02*	22.60
IV.	MKCF	100	0.34±0.02*	35.84
V.	FLPE	100	0.13±0.01***	69.10
VI.	FLEA	100	0.41±0.02 *	22.64
VII.	FLCF	100	0.40±0.07*	28.14
VIII.	FLET	100.	0.16±0.01***	68.72
IX	Pyrilamine	1mg/kg	0.09±0.01***	78.10

MEV Values represent Mean± SEM; MEV = Mean Edema volume & PI = Percentage inhibition; Group I- Edema Control (saline); Group II- Arthritic rats treated with MKPE fraction, Group III- Arthritic rats treated with MKEA fraction, Group IV- Arthritic rats treated MKCF fraction from *M. koenigii*, Group V - Arthritic rats treated with FLPE fraction, Group VI- Arthritic rats treated with FLEA fraction, Group VII- Arthritic rats treated with FLCF fraction, Group VIII- Arthritic rats treated with FLET fraction from *Ficus lacor*, Group IX- Arthritic rats treated with Pyrilamine 1mg/kg. *p<0.05, **p<0.01, *** p <0.001 as compared to arthritic control.

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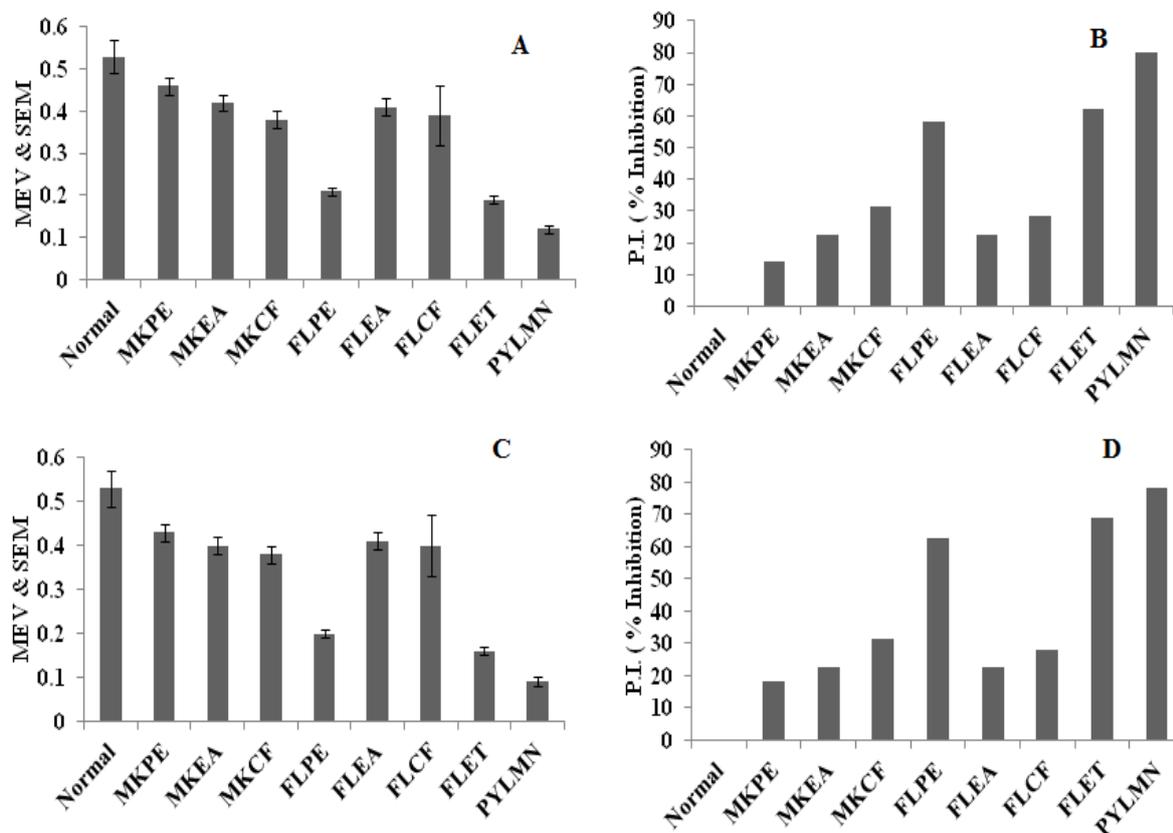


Fig. 3 Inhibition of Serotonin induced paw edema in rats by different extracts from *Murraya koenigii* and *Ficus lacor*, A and B Shows at lower dose and C and D at higher doses

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