

**EVALUATION OF ANTI-INFLAMMATORY
ACTIVITY OF METHANOL EXTRACT OF
MURRAYA KOENIGI LEAVES BY *IN VIVO*
AND *IN VITRO* METHODS**

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Summary

The methanol extract of *Murraya Koenigi* leaves (MMK) was evaluated for anti-inflammatory activity using *in vivo* and *in vitro* methods. In acute inflammation tests, like carrageenan, histamine and serotonin induced rat paw edema, MMK produced significant inhibition in dose dependent manner. Also significantly reduced castor oil induced diarrhoea and acetic acid-induced vascular permeability in mice. In the chronic inflammation experiment, MMK significantly inhibited cotton pellet-induced granuloma in rat. The MMK exhibited significant membrane-stabilizing property when red blood cells (RBC's) were exposed to hypotonic solution. Thermal induced protein denaturation was significantly inhibited by the extract. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's t-tests. In conclusion, MMK demonstrated anti-inflammatory activity in *in vivo* and *in vitro* methods suggesting its putative role in therapeutics.

Key words: Inflammation, acute, chronic, membrane stabilization, protein denaturation.

Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [1]. Inflammation however, if runs unchecked, leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis [2]. It is believed that current drugs available such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases of inflammatory disorders, because of their side effects and potency [3]. As a result, a search for other alternatives is necessary. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries have yielded compounds for the development of modern drugs [4].

Murraya Koenigi (L.) Spreng (Family Rutaceae) commonly known as curry leaf is a small strong smelling perennial shrub or small tree. The plant usually cultivated for its aromatic leaves is normally used for natural flavouring in curries and sauces. This plant is distributed in India, Malaysia, South Africa, Andaman Islands and throughout Central and Southeast Asia [5]. Parts of the plant have been used as raw material for traditional medicine formulation in India [6]. This species is known to possess anti-inflammatory, antidiabetic, antioxidant, antidiabetic and diverse pharmacological properties [7]. The reported activities of *Murraya Koenigi* (*M. Koenigi*) are antibacterial, antidiabetic, antioxidant and hypolipidemic activity [8]. *M. Koenigi* contains carbazole alkaloids namely murrayanine, mahanimbine, girinimbine, murrayacine, isomurrayazoline, mahanine, koenine, Koenigine, koenidine, koenimbine and 8,8'-bisKoenigine [8]. Carbazole alkaloids present in the fresh leaves of *M. Koenigi* showed significant inhibition of hPGHS-1 and hPGHS-2 when tested *in vitro* in antiinflammatory assays [9]. Essential oil present in the leaves of *M. Koenigi* has been studied by various researchers. But there is no systematic scientific evidence to show the utility of *M. Koenigi* leaves extract in the inflammatory conditions and about its mechanism of action.

Thus, the present study has been planned to investigate the anti-inflammatory effect of methanol extract of *M. Koenigi* leaves and to find out their mechanism of action by using different *in vivo* and *in vitro* methods of inflammation. Each experimental method tested represents different phases of inflammation.

Materials and Methods

Plant material

Fresh leaves of the plant *M. Koenigi* (L.) Spreng (Family-Lauraceae) were collected from Mumbai region, India. The plant material was taxonomically identified by Dr. Ganesh Iyer, Prof. in Botany, Ramnarain Ruia college, Mumbai, India. A voucher specimen (No. 16-04/08) has been preserved in our laboratory for future reference. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container.

Plant extraction

The dried powder material of the leaves was defatted with petroleum ether (60⁰-80⁰ C) and subsequently extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and methanol extract of *M. Koenigi* leaves (MMK) was obtained (yield 8.6%). The extract was stored at 4⁰C in a sealed vials till required. Suspension of MMK was prepared freshly in (0.5%w/v) Sodium Carboxymethylcellulose (NaCMC) and used as dosage in animal studies.

Animal

Wistar albino rats of either sex weighing 180–200 g and Swiss albino mice of either sex weighing 18–22 g were used for animal studies. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) and relative humidity (50±5%) with dark and light cycle (14/10 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats and mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The Institutional Animal Ethics Committee had approved the experimental protocols and care of animals was taken according to CPCSEA guidelines.

Chemicals and drugs

Carrageenan (Sigma Aldrich, USA), Histamine, Serotonin, Egg albumin and Evans' blue were obtained from Himedia Lab [(Mumbai), India], Indomethacin [Recon, (Bangalore) India], Ascorbic acid (Vit C) [Raj enterprises, (Mumbai) India]. Cyproheptadine [Fleming Laboratories Limited, (Hydrabad), India], Castor oil [Jayant Agro Organics Limited, (Mumbai), India], all other chemicals used were of analytical grade.

Phytochemical screening

The MMK extract was screened for the presence of various phytochemical constituents i.e. steroids, alkaloids, tannins, flavonoids, glycosides etc, by employing standard screening test [10].

Acute toxicity study

Acute toxicity study was performed as per OECD-423 guidelines [11]. Swiss albino mice of either sex were used. The animals were fasted for 6 h, but allowed free access to water *ad libitum* throughout. The animals were divided into seven groups containing six animals each. MMK was suspended in (0.5% w/v) NaCMC and administered orally as a single dose to mice at different dose levels viz. 500, 750, 1000, 1250, 1500 and 2000 mg/kg of body weight (b.w.). The control group received a similar volume of (0.5 % w/v) Na CMC solution (1 ml/ 100 gm). Mice were observed periodically for 24 hours and then daily for next 14 days.

Anti-inflammatory Activity

Carrageenan-induced rat paw edema

This test was followed by the method described by Winter *et al* [12]. Rats were divided into five different groups (n = 6). Group I served as control and received the vehicle [(0.5% w/v) NaCMC, 5 ml/kg]. Group II, III and IV received MMK extract at the dose levels of 100, 200 and 400 mg/kg orally. Group V received indomethacin at a dose of 10 mg/kg. One hour after the respective treatment, 100 μ l of 1% freshly prepared carrageenan in normal saline was injected in sub-plantar region of right hind paw of rats. The paw volume was measured at 0 h i.e. immediately after carrageenan injection and then at 1, 2, 3 and 4 h using plethysmometer.

The anti-inflammatory effect of MMK was calculated by the following equation:

Anti-inflammatory activity (%) Inhibition = $(1-D/C) \times 100$,

Where D represents the percentage difference in paw volume after the administration of test drugs to the rats and C represents the percentage difference of volume in the control groups [13].

Histamine and serotonin-induced rat paw edema

The paw edema was produced by subplantar administration of 0.1 ml of a 0.1% freshly prepared solution of histamine or serotonin into the right hind paw of rats. The paw volume was recorded at 0 and 1 h after histamine injection [14]. Different groups of animals were pretreated with MMK (100, 200 and 400 mg/kg) or with 5 ml/kg of 0.5 % (w/v) NaCMC or 10 mg/kg cyproheptadine (standard drug). The drugs were administered orally 1 h before eliciting paw edema. Percent inhibition of paw edema was calculated as described in carrageenan induced rat paw edema method.

Castor oil induced diarrhoea in mice

This test was followed by the method described by Awouters et al [15] with some modification. Five groups of six mice per group were used. The mice were starved for 10 h prior to the experiment. Group I was treated with 1 ml/100 gm NaCMC [0.5 % (w/v)] (vehicle control group) orally, groups II, III and IV were treated with 100, 200 and 400 mg of MMK extract/kg respectively while group V received 10 mg indomethacin/kg orally. One hour after the treatment, mice in all the groups were given 0.1 ml castor oil/10 g b.w. orally. The mice in each group were then placed singly in cages having adsorbent paper beneath and examined for the presence and frequency of wet stool every hour for 4 h. Absence or delay in production of watery stool was regarded as protective or positive.

Acetic acid-induced vascular permeability in mice

This test was done according to the method described by Whittle [16] with minor modification. Five groups of six mice each were used. Group I treated as vehicle control, groups II, III and IV were treated with 100, 200 and 400 mg MMK extract/kg orally respectively, while group V received indomethacin 10 mg /kg orally.

One hour after the treatment, 0.2% Evan's blue in normal saline was injected intravenously through tail vein at a dose of 0.1 ml/10 g b.w. Thirty minutes later, each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. After 1 h, the mice were sacrificed and the abdominal wall was cut to expose the entrails. The abdominal cavity was washed using 5 ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetric analysis using a spectrophotometer at a wavelength of 590 nm. The vascular permeability effects were expressed as the absorbance (A), which represented the total amount of dye leaked into the intraperitoneal cavity.

Cotton pellet-induced granuloma in rats

The cotton pellets induced granuloma in rats was studied according to the method described by D'Arcy *et al* [17]. The animals were divided into five groups of six animals each. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle [(0.5% w/v) NaCMC, 5 ml/kg]. The extract MMK at the dose levels of 100, 200 and 400 mg/kg was administered orally to groups II, III and IV respectively for seven consecutive days from the day of cotton pellet implantation. Group V received indomethacin at a dose of 10 mg/kg for the same period. On 8th day the animals were anaesthetized and the pellets together with the granuloma tissues formed were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60 °C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the weight of the pellets was taken as a measure of granuloma formation and compared with control.

Membrane stabilizing activity

This test was done according to the method described by Shinde *et al* [18] with some modifications. Whole human blood was obtained from healthy human volunteer and transferred to heparinized centrifuge tube. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) for 10 minutes at 3000g

The test sample consisted of stock erythrocyte (RBC) suspension (0.5 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the MMK extract (0.2- 1.0 mg/ml) or indomethacin (0.1 mg/ml). The control sample consisted of 0.5 ml of RBC suspension mixed with hypotonic buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000g and the absorbance of the supernatant was measured at 540 nm. Each experiment was done in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times [A_1 - A_2 / A_1].$$

Where:

A₁ = Absorption of hypotonic-buffered saline solution alone

A₂ = Absorption of test sample in hypotonic solution

Inhibition of protein denaturation

Test solution (1ml) containing different concentrations (25 - 250 µg/ml) of MMK extract or indomethacin (100 µg/ml) was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ± 1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling the turbidity was measured spectrophotometrically at 660 nm [19]. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken.

Statistical analysis

The experimental data was expressed as mean ± SEM, the significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's t-test using Graphat Instat Software (San Diego, CA, USA).

Results

Phytochemical Screening

Preliminary phytochemical screening of MMK revealed the presence of steroids, tannins, flavonoids, alkaloids and glycosides. Further separation of the specific phytochemicals is in progress.

Acute Toxicity Test

In the acute toxicity assay no deaths were observed during the period of the study at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis. The median lethal dose (LD50) was determined to be higher than highest dose tested i.e. 2.0 g/ kg b.w.

Anti-inflammatory Activity

Carrageenan-induced rat paw edema

Table 1 shows that injection of carrageenan in control group induced acute inflammation with a prominent increase in paw volume, began 1 h after intraplantar injection and reached a peak of inflammation after 4 h. Pretreatment of animals with the different doses of MMK (100, 200 and 400 mg/kg) caused inhibition on carrageenan induced inflammation dose dependently. MMK at the doses 100 and 200 mg/kg inhibited moderately paw edema (25.65 and 40.13%), whereas at the dose of 400 mg/kg and indomethacin at dose of 10 mg/kg inhibited significantly ($p<0.05$) (58.55 and 69.07 % respectively) after 4 h of carrageenan injection when the results were compared to that of control group.

Histamine and serotonin-induced rat paw edema

The MMK extract (100, 200 and 400 mg/kg) significantly ($p<0.01$) and dose dependently inhibited histamine-induced rat paw edema (15.49, 26.32 and 40.93%, respectively) and cyproheptadine (10 mg/kg) produced inhibition of 55.08% when compared with control group after 1 h of histamine injection (Table 2). Also the MMK extract (100, 200 and 400 mg/kg) dose dependently inhibited serotonin-induced rat paw edema. CTM 400 mg/kg exhibited maximum inhibition of 38.27% in serotonin-induced rat paw edema whereas cyproheptadine produced inhibition of 51.48% after 1 h of the serotonin injection (Table 2).

Castor oil induced diarrhoea in mice

The MMK extract inhibited castor oil-induced diarrhoea in mice in a dose dependent manner producing maximal inhibition at 400 mg/kg. MMK at the dose of 400 mg/kg and indomethacin at 10 mg/kg

significantly ($p<0.01$) protected (83% and 100% respectively) mice against castor oil-induced diarrhoea (Table 3).

Acetic acid-induced vascular permeability in mice

Effect of MMK (100, 200 and 400 mg/kg), indomethacin (10 mg/kg) and control vehicle on acetic acid-induced increased vascular permeability in mice was studied. Results of the study showed that MMK at dose of 100 and 200 mg/kg moderately inhibits the vascular permeability (27.53% and 38.60% respectively). Where as CTM at dose of 400 mg/kg and indomethacin 10 mg/kg significantly ($p<0.01$) inhibits vascular permeability (60.02% and 68.45% respectively) when compared with vehicle control (Table 4).

Cotton pellet-induced granuloma in rats

In the model of chronic inflammation using the cotton pellet-induced granuloma in rats, the MMK inhibited significantly ($p<0.01$) the formation of granulomatus tissues in a dose-dependent manner (Table 5). MMK at dose 400 mg/kg showed 63.40% (wet) and 55.76% (dry) inhibition of granuloma formation, whereas indomethacin 10 mg/kg showed 68.04% (wet) and 59.61% (dry) inhibition of granuloma formation.

Membrane stabilizing activity

The MMK extracts at concentration range of 0.6-1.0 mg/ml and indomethacin (0.10 mg/ml) protected significantly ($p<0.01$) the erythrocyte membrane against lysis induced by hypotonic solution. The MMK extract at a concentration of (1 mg/ml) and indomethacin at concentration of 0.1 mg/ml showed 70.41% and 54.79% respectively inhibition of RBC haemolysis (Table 6).

Protein denaturation

The inhibitory effects of different concentrations of MMK on protein denaturation are shown in Table 7. MMK at concentration level of 50- 250 $\mu\text{g/ml}$ showed significant ($p<0.05$) inhibition of denaturation of egg albumin in concentration depended manner. The MMK extract at a concentration of 250 $\mu\text{g/ml}$ and indomethacin at concentration of 100 $\mu\text{g/ml}$ showed 52.38% and 84.61% respectively inhibition of protein denaturation.

Table 1: Effect of methanol extract of *M. Koenigi* leaves on carrageenan induced rat paw edema.

Treatment (s)	Dose (mg/kg)	Increased in paw volume (percentage inhibition) in mL				
		0h	1h	2h	3h	4h
Control	Vehicle	0.21± 0.030	0.285 ± 0.026	0.375 ± 0.022	0.433 ± 0.018	0.463 ± 0.019
Methanol extract of <i>M. Koenigi</i> leaves	100	0.205± 0.024	0.268 ± 0.018 (15.52)	0.340 ± 0.022 (18.16)	0.378 ± 0.024 (22.38)	0.393 ± 0.021 (25.65)
	200	0.203 ± 0.23	0.265 ± 0.019 (17.67)	0.318 ± 0.016 (30.28)	0.345 ± 0.02 (36.57)	0.355 ± 0.023 (40.13)
	400	0.205±0.021	0.260±0.027 (26.66)	0.292±0.013* (48.39)	0.301±0.017* (56.71)	0.310±0.024* (58.55)
Indomethacin	10	0.203±0.022	0.230±0.023* (62.54)	0.256±0.019** (65.12)	0.275±0.021** (67.91)	0.281±0.018** (69.07)

Each value represents the mean ± S.E.M., n=6. * p<0.05; ** p<0.01 compared with control, Dunnett's *t*-test after analysis of variance. Values in parenthesis represent percent inhibition of paw edema.

Table 2: Effect of methanol extract of *M. Koenigi* leaves on histamine and serotonin induced rat paw edema

Treatment (s)	Dose (mg/kg)	Percentage increase in swelling	Percentage of inhibition
Histamine control	Vehicle	51.16 ± 1.72	-----
Cyproheptadine (standard)	10	22.97 ± 1.67**	55.08
Methanol extract of <i>M. Koenigi</i> leaves	100	43.23 ± 1.21*	15.49
	200	37.69 ± 1.85**	26.32
	400	30.21 ± 1.07**	40.93
Serotonin control	Vehicle	46.15 ± 2.02	-----
Cyproheptadine (standard)	10	22.39 ± 1.95**	51.48
Methanol extract of <i>M. Koenigi</i> leaves	100	40.47 ± 1.74*	12.30
	200	35.69 ± 1.48**	22.66
	400	28.48 ± 1.15**	38.27

Each value represents the mean ± S.E.M., $n=6$.

* $p<0.05$; ** $p<0.01$ compared with control, Dunnett's t -test after analysis of variance.

Table 3: Effect of methanol extract of *M. Koenigi* leaves on castor oil-induced diarrhoea in mice

Treatment (s)	Dose (mg/kg)	No. of mice protected from diarrhoea (% Inhibition)			
		1h	2h	3h	4h
Control	Vehicle	0	0	0	0
Methanol extract of <i>M. Koenigi</i> leaves	100	1 (17%)	3 (50%)	4 (67%)	4 (67%)
	200	2 (33%)	3 (50%)	4 (67%)	5 (83%)
	400	4 (67%)	5 (83%)	5 (83%)	5 (83%)
Indomethacin	10	6 (100%)	6 (100%)	6 (100%)	6 (100%)

Each value represents the mean \pm S.E.M., $n=6$.

Table 4: Effect of methanol extract of *M. Koenigi* leaves on acetic acid-induced vascular permeability in mice

Treatment (s)	Dose (mg/kg)	Absorption at 590 nm	% Inhibition
Control	Vehicle	0.158	-----
Methanol extract of <i>M. Koenigi</i> leaves	100	0.1114±0.31*	27.53
	200	0.097±0.78**	38.60
	400	0.060±0.24**	62.02
Indomethacin	10	0.049±0.75**	68.45

Each value represents the mean ± S.E.M., $n=6$.

* $p<0.05$; ** $p<0.01$ compared with control, Dunnett's t -test after analysis of variance.

Table 5: Effect of methanol extract of *M. Koenigi* leaves on cotton pellet-induced granuloma in rats

Treatment (s)	Dose (mg/kg)	Weight of granulation (mg) (wet)	Percentage inhibition	Weight of granulation (mg) (dry)	Percentage inhibition
Control	Vehicle	194 ± 7.2	-----	52 ± 8.4	-----
Methanol extract of <i>M. Koenigi</i> leaves	100	157 ± 7.8*	19.07	42 ± 4.7*	19.23
	200	106 ± 8.0**	45.36	30 ± 3.6**	42.30
	400	71 ± 7.3 **	63.40	23 ± 3.2**	55.76
Indomethacin	10	62 ± 6.1**	68.04	21 ± 3.4**	59.61

Each value represents the mean ± S.E.M., $n=6$.

* $p<0.05$; ** $p<0.01$ compared with control, Dunnett's t -test after analysis of variance.

Table 6: Effect of methanol extract of *M. Koenigi* leaves on erythrocyte heamolysis

Sample (s)	Concentration	Absorption at 540 nm	% Inhibition of haemolysis
Hypotonic medium	50 mM	0.730 ± 0.06	-----
Methanol extract of <i>M. Koenigi</i> leaves	0.2 mg/ ml	0.569 ± 0.5	22.05
	0.4 mg/ ml	0.514 ± 0.37*	29.58
	0.6 mg/ ml	0.385 ± 0.16**	47.26
	0.8 mg/ ml	0.312 ± 0.29**	57.26
	1 mg/ ml	0.216 ± 0.41**	70.41
Indomethacin	0.1 mg/ ml	0.330 ± 0.24**	54.79

Each value represents the mean ± S.E.M, n=3

* $p < 0.05$; ** $p < 0.01$ compared with to blank, Dunnett's *t*-test after analysis of variance.

Table 7: Effect of methanol extract of *M. Koenigi* leaves on protein denaturation

Sample (s)	Concentration	% Inhibition of protein denaturation
Vehicle control	Vehicle	-----
Methanol extract of <i>M. Koenigi</i> leaves	50 µg/ ml	14.28
	100 µg/ ml	26.19
	150 µg/ ml	35.71
	200 µg/ ml	40.47
	250 µg/ ml	52.38
Indomethacin	100 µg/ ml	84.61

Each value represents the mean \pm S.E.M., $n=3$.

Discussion

The present study establishes the anti-inflammatory activity of the methanol extract of MMK in a number of *in vivo* and *in vitro* methods, representing different phases of inflammation. Results of the present experiment demonstrate that MMK play significant role in the inhibition of inflammatory process by acting on different phases of inflammation.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation study and is believed to be biphasic. The early phase (1–2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The later phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [20]. The inhibitory activity shown by the MMK at 400 mg/ kg over a period of 4 h in carrageenan-induced paw inflammation was quite similar to that exhibited by the group treated with indomethacin. These results indicate that the extract at higher dose level acts significantly on both early and later phases. In the later phase probably involving arachidonic acid metabolites, which produce an edema dependent on neutrophils mobilization [14].

The early phase of inflammation mainly mediated by histamine and serotonin. Significant inhibition of histamine and serotonin induced rat paw edema by MMK extract revealed that the anti-inflammatory activity is possibly backed by its anti-histamine and anti-serotonin activity.

The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa. In human, prostaglandins cause intestinal cramps and diarrhoea which is due to effect on intestinal smooth muscle and secretion. Ricinoleic acid, the active principle in castor oil caused changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to hyper-secretory response and diarrhoea.

It causes irritation and inflammation of the intestinal mucosa, leading to prostaglandin release, which results in an increase in the net secretion of water and electrolytes into the small intestine. The inherent resting tone of the intestinal smooth muscle is maintained by continuous intramural generation of prostaglandins and the inhibition of PG biosynthesis resulted in decrease of the resting tone. Inhibitors of prostaglandin biosynthesis were observed to delay castor oil-induced diarrhoea. MMK showed significant reduction in the castor oil induced diarrhoea indicating that the extract has the ability to inhibit the synthesis of prostaglandins.

Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid [21]. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (acetic acid) causes an immediate sustained reaction that is prolonged over 24 h [22] and its inhibition suggests that the MMK extract may effectively suppress the exudative phase of acute inflammation.

Cotton pellet granuloma is a chronic inflammation reaction arising when the acute response is insufficient to eliminate proinflammatory agents. It occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The cotton pellet method is widely used to evaluate the transudative (infiltration of neutrophils and exudation) and proliferative (proliferation of fibroblasts) components of the chronic inflammation [21, 23]. The wet weight of the cotton pellets correlates with the transudate and the dry weight of the pellets correlates with the amount of the granulomatous tissue [24, 25]. Administration of MMK (100, 200 and 400 mg/kg) appear to be effective in inhibiting both wet and dry weight of the cotton pellet in dose dependent manner. MMK showed significant ($p < 0.01$) anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts, synthesis of collagen and mucopolysaccharides during granuloma tissue formation [26].

The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin [27, 28]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation [27, 28]. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances [29, 30]. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators [31]. However, the MMK has shown significant ($p < 0.01$) membrane stabilizing property, which suggests that its anti-inflammatory activity observed in this study may be by prevention of the release of phospholipases that trigger the formation of inflammatory mediators.

Denaturation of proteins is a well documented cause of inflammation and rheumatoid arthritis [32]. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [33]. Ability of MMK to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity.

The anti-inflammatory effect of MMK observed in above methods tested may be due to the presence of Carbazole alkaloids [8] in the plant extract. It has been reported that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities [34]. Thus, the methanol extract of *M. Koenigi* leaves produces significant anti-inflammatory activities in dose dependent manner on both acute and chronic animal models and *in vitro* methods of inflammation.

Conclusion

The data obtained from our current study indicated that several factors may contribute to the anti-inflammatory action of MMK. First, potent inhibition of rat paw edema and castor oil induced diarrhoea in mice shows inhibition of prostaglandins synthesis is a major mechanism by which the plant extract exerts anti-inflammatory activity. Second, MMK significantly inhibited histamine and serotonin induced rat paw edema showing its anti-histaminic and anti-serotonin ability. Third, MMK reduced the increased peritoneal vascular permeability in mice, indicating the suppression of the vascular response in the process of acute inflammation. Fourth, in the chronic inflammation model MMK reduced the formation of granuloma tissue in a dose-dependent manner, which represented an ability to inhibit the proliferation phase of inflammatory process. Fifth MMK exhibit significant membrane-stabilizing property and inhibition of protein denaturation.

Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

Acknowledgement

This work was supported by grants from University Grants Commission, India. The authors gratefully acknowledge the financial assistance and fellowship from University Grants Commission, India.

References

- 1) Denko CW. A role of neuropeptides in inflammation. In: Whicher JT, Evans SW, eds. *Biochemistry of Inflammation*, ed. London: Kluwer Pub, 1992:177-181.
- 2) Henson PM, Murphy RC. *Mediators of the inflammatory process*. Amsterdam Elsevier, 1989: p 404.

- 3) Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S. Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. *J Ethnopharmacol* 1998;61:229.
- 4) Arivazhagan S, Balasenthi S, Nagini S. Antioxidant and antiinflammatory activities of *Mallotus oppositifolium*. *J Phytother Res* 2000;14:291-293.
- 5) Sastri BN. *The Wealth of India, A Dictionary of India Raw Material and Industrial Products*. CSIR, New Delhi: India, 1952.
- 6) Chowdhury JJ, Bhuiyan NI, Yusuf. Chemical composition of the leaf essential oils of *Murraya Koenigii* (L.) Spreng and *Murraya paniculata* (L). *Bangladesh J Pharmacol* 2008; 3: 59-63.
- 7) Drury HC. *The useful plants of India*. Allen: London, 1978 pp 78.
- 8) Iyer D, Uma D. Phyto-pharmacology of *Murraya Koenigii* (L). *Pharmacognosy reviews* 2008; 2 (3):180-184.
- 9) Russel SR, Muraleedharan GN, Gale MS, David LD, John LN. Biologically Active Carbazole Alkaloids from *Murraya Koenigii*. *J Agric Food Chem* 1999;47(2):444-447.
- 10) Trease GE, Evans MC. *Text book of Pharmacognosy*. 12th edn. London: Balliere, Tindall; 1983.
- 11) Ecobichon DJ. *The Basis of Toxicology Testing*. New York: CRC Press; 1997.
- 12) Winter CA, Porter CC. Effect of alteration in side chain upon antiinflammatory and liver glycogen activities in hydrocortisone ester. *J Amer Pharmacol Soci* 1957;46:515-519.
- 13) Suleyman H, Demirezer LO, Kuruuzum A, Banoglu ZN, Gocer F, Ozbakir G, Gepdiremen A. Antiinflammatory effect of the aqueous extract from *Rumex patientia* L. roots. *J Ethnopharmacol* 1991;65:141-148.
- 14) Just MJ, Recio MC, Giner RM, Cullar MJ, Manez S, Bilia AR. Antiinflammatory activity of unusual Lupane saponins from *Bupleurum fruticosens*. *Plant Med* 1998;64:404-407.
- 15) Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA. Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *J Pharm. Pharmacol* 1978; 30: 41-45.
- 16) Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. *Br J Pharmacol* 1964;22:246-253.

- 17) D'Arcy PF, Haward EM, Muggleton RW, Townsend SB. The anti-inflammatory action of griseofulvin in experimental animals. *J Pharm Pharmacol* 1960;12:659-665.
- 18) Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO. Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* 1999;70:251-257.
- 19) Elias G, Rao MN. Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. *Indian J Exp Biol* 1988;26:540-542.
- 20) Brito ARMS, Antonio MA. Oral antiinflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). *J Ethnopharmacol* 1998; 61:215-228.
- 21) Brown JN, Roberts J. Histamine, bradykinin, and their antagonists. In: Gilman AG, Hardman JG, Limbird LE, eds. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 10th edn. New York: McGraw Hill Co, 2001: 645–667.
- 22) Whaley K, Burt AD. Inflammation, healing and repair. In: MacSween, R.M.N, Whaley K, eds. *Muir's Textbook of Pathology*, 13th edn. London: Arnold, 1996: 112–165.
- 23) Arrigoni-Maratellie E. *Inflammation and Antiinflammatory*, Spectrum. New York: Publication Inc, 1988.
- 24) Olajide OA, Awe SO, Makinde JM. Effect of the aqueous extract of *Bridelia ferruginea* stem bark on carrageenan-induced edema and granuloma tissue formation in rats and mice. *J Ethnopharmacol* 1999;66:113-117.
- 25) Olajide OA, Awe SO, Makinde JM, et al. Studies on the antiinflammatory, antipyretic and analgesic properties of the *Alstonia boonei* stem bark. *J Ethnopharmacol* 2000; 71:179-186.
- 26) Recio MC, Giner RM, Manez S, Ros JL. Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Planta Med* 1995;6:182-185.
- 27) Augusto O, Kunze KL, Montellano PR. Nphenylprotoporphyrin formation in the haemoglobinphenylhydrazine reaction. *J Biol Chem* 1982;257:6231-6241.

- 28) Ferrali M, Signorni C, Ciccoli L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenylhydrazine, divicine and isouramil. *Biochem J* 1992;285:295-301.
- 29) Maxwell SRJ. Prospects for the use of anti-oxidant therapies. *Drugs* 1995;49:345-361.
- 30) Liu GT, Zhang TM, Wang BE, Wang YW. Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. *Biochem Pharmacol* 1992;43:147-152.
- 31) Aitadafoun M, Mounieri C, Heyman SF, Binistic C, Bon C, Godhold J. 4-Alkoxybenzamides as new potent phospholipase A2 inhibitors. *Biochem Pharmacol* 1996; 51:737-742.
- 32) Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol* 1968;20:169-173.
- 33) Grant NH, Alburn HE, Kryzanasuskas C. Stabilization of serum albumin by anti-inflammatory drugs. *Biochem Pharmacol* 1970;19:715-722.
- 34) Fiebig M, Pezzutp JM, Soejartp DD, Kinghorn AD. Koeline, A further cytotoxic carbazole alkaloids from *Murraya Koenigii*. *Phytochemistry* 1985;24: 3041-3043.