© 2018 IJSRST | Volume 4 | Issue 8 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Anti-Inflammatory Activity of Alkaloids from Murraya Koenigii Leaves In Animal Models

Rupali Patil

Department of Pharmacology, Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Education & Research, Nasik, India

ABSTRACT

Alkaloids have a wide range of pharmacological properties, including anti-inflammatory activity. The purpose of the present study was to investigate the anti-inflammatory and antioxidant activity of *Murraya koenigii* leaves. The hind paw edema was produced in rats by subplantar injection of Carageenan. Pet ether extract (PMK) of *Murraya koenigii* leaves and alkaloids (AMK) isolated from PMK at doses of 100 and 300 mg/kg/day, p.o. were given for 11days to observe % inhibition of paw edema which was comparable with Aspirin (100 mg/kg, p.o.) used as a reference drug. PMK and AMK produced a significant ($p<0.05$) inhibition of paw edema. PMK and AMK treatment significantly reversed the Carageenan induced decrease in paw Superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH) levels as compared to Carageenan treated rats. Lipid peroxidation (LPO) induced by Carageenan treatment was significantly reversed after administration of PMK and AMK. hematological analysis of carageenan-treated rats exhibited significant ($p<0.05$) decrease in RBC count, Hb content and PCV after treatment with aspirin, compared to the control. PMK or AMK treated animals showed normal erythrocyte (RBC) count, hemoglobin (Hb), packed cell volume (PCV), near to control group. The total leukocyte (WBC), lymphocyte, neutrophils, and basophils count were higher in rats treated with aspirin compared to control. PMK and AMK treatment showed decreased platelet count compared to control. The significant reductions observed in the activity of ALT and AST in PMK and AMK treated animals compared to control.

Keywords : Inflammation, Carbazole Alkaloid, Carageenan, *Murraya Koenigii*

I. INTRODUCTION

Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective insults which frequently and erroneously leads to the damaging of normal tissues (Serhan and Levy 2003). The process of inflammation is characterized by increased vascular permeability at inflamed site followed by localization and margination of neutrophils. An acute inflammatory process is comprised of inflammation mediators including neutrophil-derived reactive oxygen species (ROS), nitric oxide (NO^-) (Syahida et al. 2010; Valko et al. 2006), prostaglandins (PGs), and cytokines (FitzGerald and Patrono 2001). Oxidative mechanisms are reported at the origin of inflammation and ROS such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues and has suggested the use of antioxidant substances (Trenam 1992; Bermond 1989). Therefore, compounds that have scavenging activities toward these radicals and/or suppressive activities on lipid peroxidation may be expected to have therapeutic potentials for several inflammatory diseases (Serhan and Levy 2003).

NSAIDs, including cyclooxygenase (COX)-2 inhibitors, exhibit anti-inflammatory effect through inhibition of COX and are among the most widely prescribed medications for the clinical treatment of inflammatory diseases such as arthritis, lumbago and rheumatism. However, NSAIDs have been associated with gastrointestinal (GI) toxicity. The cardiovascular (CV) toxicity is associated with COX-2 inhibitors (Scheiman and Hindley 2010). Numerous clinical observations have associated the use of aspirin with blood disorders like anemia and cytopenias (Raybak, 1992). In this context, there arise new scopes for Herbs and Herbal Formulation in treatment of inflammatory disorders.

Murraya koenigii L. (Rutaceae), commonly known as curry tree, is a tropical to sub-tropical tree native to India. Traditionally the plant is used as tonic, stomachic and carminative (Kirtikar and Basu 1993). Methanolic extract of *Murraya koenigii* leaves is known to possess analgesic and anti-inflammatory activity (Gupta *et al*. 2010). *Murraya koenigii* contains carbazole alkaloids (Narasimhan et al.1968; Chowdhury and Chakraborty 1971) having antioxidant (Rao *et al*. 2006, Arulselvan and Subramanian 2007; Gupta and Sharma 2010) anti-inflammatory, anti-tumour (Muthumani *et al*.2009), anti-trypanocidal (Das and Chakraborty 1965) and mosquitocidal activities (Chakrabarty *et al*.1997).

The present research was aimed to evaluate the anti-inflammatory and antioxidant activity of pet ether extract of *Murraya koenigii* L. leaves (PMK) and total alkaloids separated from pet ether extract of *Murraya koenigii* leaves L. (AMK). The anti-inflammatory activity of PMK and AMK was evaluated using carrageenan induced rat paw edema (Winter et al. 1962). The antioxidant activity revealed by in vitro radical scavenging assays is further confirmed by evaluating cellular antioxidant defense system. The study was further extended to assess effect of PMK and AMK on haematological profile of experimental animals.

II. METHODS AND MATERIAL

1) Animals

Albino Wistar rats (100-150 g) of either sex were used for this study. The animals were housed at 24 ± 2°C and relative humidity 55 ± 5 with 12:12 h light and dark cycle. They had free access to food and water ad libitum. The animals were acclimatizated for a period of seven days before the study. The experimental protocol was approved *by the* Institutional Animal Ethics Committee (IAEC) of MGV's Pharmacy College, Nasik.

2) Drugs and chemicals

Carrageenan and 1, 1-diphenyl-2-picryl hydrazyl (DPPH) (Sigma-Aldrich, MO, USA), aspirin (Research Lab, Mumbai) were used for this study.

3) Plant material and extraction

Leaves of *Murraya koenigii* (1 Kg) were purchased from the local market and were identified by Dr. P.G. Diwakar, Jt. Director, Botanical Survey of India, Pune, where a voucher specimen (MUKKID 1) has been retained. The leaves were dried in shade and powdered mechanically. Powdered leaves of Murraya koenigii were defatted with the Petroleum ether (60-80°C). The filtrate was concentrated to get pet ether extract of Murraya koenigii (PMK) (yield: 12.6 % w/w).

The extract was further subjected to isolation of alkaloids according to method of Cordell GA (1981). The aqueous portion was dried to get crude alkaloidal fraction of pet ether extract of Murraya koenigii leaves (AMK) (yield: 48.33 % w/w).

4) Treatment

Rats were randomly divided into 6 groups, each containing 5 animals. The previous studies carried out in our lab revealed that PMK and AMK

administration for 11 days exhibit highest antinociceptive activity. Accordingly, PMK (100 and 300 mg/kg) was suspended in 0.1 % CMC and AMK (100 and 300 mg/kg) was dissolved in water and administered orally to animals for 11 days. Control group received 0.1% CMC, p.o., for 11 days.

5) Phytochemical analysis

Phytochemical analysis of PMK was carried out according to methods described earlier (Trease and Evans, 2002).

Identification

UV-visible spectra: AMK revealed peak at 295.0, 256.50 and 246.50 nm when spectra was run using Shimadzu-2450.

FTIR: AMK depicted presence of functional groups like N-H stretch (3394.83 to 3556.85 μ) Aliphatic C-H stretch superimposed on N-H stretch (2974.33 μ), N-H bend (1610.61), C-N vibration (aromatic, secondary) (1276.92).

6) In vitro Antioxidant activity

In vitro antioxidant activity of PMK was evaluated by DPPH method (Molyneux 2004). Scavenging free radical potential was evaluated against ethanolic solution of DPPH, a stable free radical.

Total antioxidant activity was determined using ammonium thiocyanate method (Mistuda et al. 1996). Scavenging activity against H_2O_2 (Ruch et al. 1989) was tested to determine ability of PMK to inhibit the formation of hydroxyl radical.

7) Anti-inflammatory activity

On 11th day of experiment, 1 h after test drug administration, pedal inflammation was induced in rats as described by (Winter et al. 1962). A suspension of 0.1 ml of 1% Carrageenan was injected into the sub plantar tissue of right hind paw of each rat. The paw volume was measured at 0, 1, 2, 3 and 4 h using Plethysmometer (UGO Basile, Italy) (Vogel, 2002). Aspirin (100 mg/kg, p.o.) was used as reference drug.

8) Biochemical estimation in Carrrageenan treated paw tissue:

After measurement of volume, the rat paws were dissected out, immediately washed in ice-cold saline and weighed. A 10 % homogenate was prepared in 0.1 M Tris buffer, pH 7.4. The homogenate was centrifuged at 15,000 g for 20 min. The supernatant was used for estimation of SOD, Catalase, GSH and LPO. Blood was collected by cardiac puncture and used for estimation of hematological parameters like erythrocyte (RBC) count, hemoglobin (Hb), packed cell volume (PCV), total leukocyte (WBC) count, lymphocyte count, neutrophils count, basophils count, glucose, alanine aminotranferease (ALT) and aspartate aminotransferase (AST).

8.1 Superoxide dismutase

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome (Saggu et al. 1989; Misra and Fridivich 1972).The results were expressed as nmol SOD U/mg wet tissue.

8.2 Catalase

The Catalase activity assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide (Beers and Sizer, 1952). The results were expressed as catalase U/g wet tissue.

8.3 Reduced glutathione

GSH (S-glutamyl sisteinylglycine) plays an important role in protection of cells against damage from endogenous and exogenous free radicals and oxidants. (Ellman, 1959). The results were expressed as nmol GSH/g wet tissue

8.4 Extent of Lipid peroxidation

LPO as evidenced by the formation of Thiobarbituric acid reactive substances (TBARS) was measured by the method of Niehaus et al., (1968). The results were expressed as LPO nmol/g wet tissue.

9) Statistical analysis

The mean ± SEM values were calculated for each group. One-way ANOVA followed by Dunnett's multiple comparison tests were used for statistical analysis. Values of $P<0.05$ was considered statistically significant.

III. RESULTS AND DISCUSSION

Phytochemical analysis of PMK revealed presence of alkaloids, triterpenoids, flavonoids, tannins and phenols.

In vitro antioxidant study

In DPPH assay, the % scavenging activity increased with the increase in concentration of PMK. The IC_{50} value was found to be 98.5 (Fig.1). Total antioxidant activity assay and H_2O_2 assay also showed increased % scavenging activity of PMK with the increase in concentration of the extract. The IC_{50} value was found to be 101.7 ppm (Fig.2) and 99.5 ppm (Fig.3) respectively.

Anti-inflammatory activity

Carrageenan treated rats showed gradual increase in paw volume from 1-3 h while the paw volume was decreased at 4th h. Treatment with PMK and AMK (100 and 300 mg/kg/day, p.o., for 11days) significantly ($p<0.05$) inhibited the extent of carageenan induced paw edema at 1st and 3rd h. Maximum inhibition was observed with AMK (300), 71.87%, at 3rd h after carageenan administration. Aspirin exhibited 72.91% inhibition at 3rd h.

Biochemical estimations in Carrrageenan treated paw tissue

Carageenan treated rats showed decreased levels of SOD, CAT and GSH in paw tissue homogenates. Administration of PMK and AMK (100 and 300 mg/kg/day, p.o., for 11days) significantly reversed the Carageenan induced decrease in paw SOD, CAT and GSH levels as compared to Carrageenan treated rats. On the other hand, Carrageenan treatment induced lipid peroxidation, as indicated by significant high MDA levels in inflamed paw tissue. Administration of PMK and AMK (100 and 300 mg/kg/day, p.o., for 11 days) significantly reversed the extent of lipid peroxidation as compared to Carrageenan treated rats.

Further, hematological analysis of carrageenan-treated rats exhibited significant ($p<0.05$) decrease in RBC count, Hb content and PCV after treatment with aspirin, compared to the control. PMK or AMK treated animals showed normal RBC count, Hb content and PCV near to control group. The WBC count, lymphocyte count, neutrophils count, basophils count were higher in rats treated with aspirin compared to control. No significant alterations were observed in blood glucose level. After treatment with PMK and AMK, platelet count was decreased compared to control.

The significant reductions observed in the activity of ALT and AST levels in PMK and AMK treated animals compared to control.

Discussion

In vitro antioxidant assay revealed that PMK has potent free radical scavenging activity. Carrageenan induced rat paw edema is a most commonly used animal model for evaluating the anti-inflammatory activity. It is believed to be biphasic (Winter et al. 1962), the first phase is due to release of histamine and serotonin (1 h), plateau phase is maintained by kinin like substance (2 h) and second accelerating phase of swelling is attributed to PG release (3 h) (Di Rosa and Willoughby 1971). It is well known that the acute

inflammatory process, in which vascular permeability increases and leukocyte migration occurs, involves inflammation mediators including neutrophil derived reactive oxygen species and free radicals, such as hydrogen peroxide, superoxide and the hydroxyl radical (Da Motta et al.1994), nitric oxide, prostaglandins (PG) and cytokines (Gualillo et al.2001). Polymorphonuclear leucocytes (Jain et al. 2001), which are the first cells to arrive at inflammatory site in the body, release free oxygen radicals ($O_2^{\cdot}$) and free hydroxyl ($OH^{\cdot}$) radicals (Mc Cord and Roy 1982). Based on this, second phase may be explained by an inhibition of cyclooxygenase or exerting of antioxidant properties (Boughton-Smith et al. 1999). As the extract exhibited significant ($P<0.05$) protection against increase in paw volume at first hour as well as third hour of carrageenan injection, it might be having Histamine, 5-Hydroxy trptamine and PG synthesis inhibitory activity.

In order to explore the effect of antioxidant defenses on the acute inflammation process, in paw tissues, the levels of SOD, CAT and GSH and extent of LPO was evaluated. Preventive antioxidants, such as SOD and CAT enzymes are the first line endogenous defense mechanism against reactive oxygen species. GSH is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated LPO (Halliwell 1995). SOD scavenges the superoxide radicals O_2, one of the ROS responsible for lipid peroxidation (Fridovich 1986). This reaction leads to increase in generation of peroxide radical H_2O_2 ·, which is also capable of producing more oxidative damage (Das et al. 1997). CAT and other peroxidases further reduce H_2O_2. Hence, increase in SOD and CAT levels result in decreased LPO levels. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of diseased states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of disulphide bonds in unsaturated lipids which eventually results in destruction of membranes. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen and metal containing fluid. Therefore, membrane lipids are susceptible to peroxidative attack (Cheeman 1993).

According to this study, administration of PMK, AMK and Aspirin showed significant ($P<0.05$) increase in the SOD, CAT and GSH levels compared to the control animals. This suggests efficacy of PMK, AMK and aspirin in preventing free radical induced damage. Similarly, a significant ($P<0.05$) inhibition of lipid peroxidation by PMK, AMK was observed which may be attributed to prevention of, a decomposed product of disrupted membrane, calculated in terms of TBARS. This further supports protective effect of *M. koenigii*. Therefore, antioxidant activity of PMK and AMK as exhibited in the *in vitro* test gets clarified.

The various biochemical and haematological parameters investigated in this study are useful indices of evaluating the toxicity of plant extract in animals (Yakubu et al. 2008). The lower RBC count, Hb content and PCV after 11 days administration of aspirin suggest that it had induced anemia associated with leucocytosis as revealed by increased WBC, lymphocyte, neutrophils and basophils count (Raybak, 1992). PMK or AMK treated animals showed normal RBC count, Hb and PCV levels near to control group suggesting that rats were not anemic. Increase in WBC, lymphocyte, neutrophils and basophils count is a normal reaction of rats to foreign substances indicating stimulation of the immune system. Aspirin, PMK and AMK exerts a significant reduction in the platelets which indicates antiplatelet property and hence the usefulness in cardiovascular diseases (Aliyu et al. 2006). The significant reductions observed in the activity of ALT and AST indicate that PMK and AMK may not be harmful to the liver.

The IR spectrum of AMK showed characteristic absorption bands in the range 3394.83 to 3556.85μ (N-

H), 2974.33 μ (C-methyls), 1610.61 μ (aromatic system) suggesting presence of pyranocarbazole nucleus. The UV spectrum of AMK showing absorption peaks at 295.0, 256.50 and 246.50 nm also supports the presence of pyranocarbazole nucleus. (Bhattacharya et al. 1982; Reisch et al.1992; Joshi et al. 1970)

The presence of carbazole alkaloids, triterpenoids and flavonoids in *Murraya koenigii* may be responsible for anti-inflammatory activity as all the three constituents have been reported to posses anti-inflammatory activity (Fernanda et al. 2004; Onasanwo and Elegbe 2000).

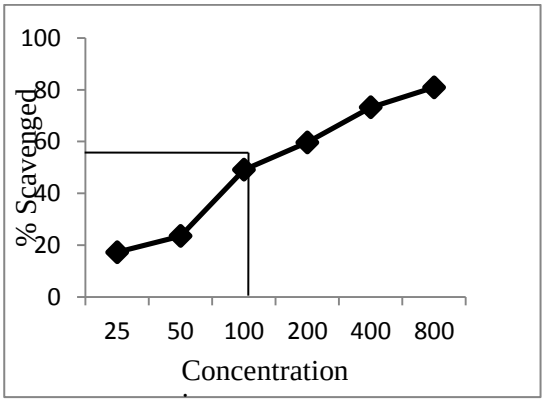

Fig.1. Free radical scavenging activity of PMK by DPPH method.

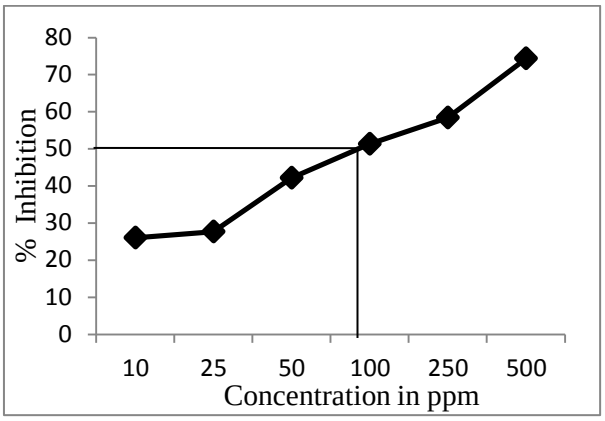

Fig.2 Total antioxidant activity of PMK by ammonium thiocyanate method.

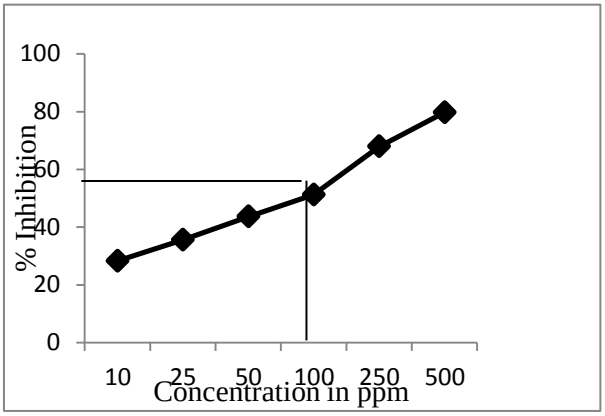

Fig.3 Hydrogen peroxide scavenging activity of PMK.

Table 1 Effect of *Murraya koenigii* on Carrageenan induced rat paw edema

Treatment (mg/kg, p.o.)	**Mean increase in paw volume (ml)**				**% Decrease in paw volume at 3rd h**
	1 h	2 h	3 h	4 h	
Control	0.31±0.02	0.76±0.02	0.96±0.04	0.72±0.06	-
Aspirin 100	0.09±0.01*	0.16±0.02*	0.26±0.02*	0.16±0.01	72.91
PMK 100	0.12±0.04*	0.26±0.06*	0.40±0.08*	0.28±0.05*	58.30
PMK 300	0.21±0.06*	0.29±0.04*	0.32±0.02*	0.36±0.10*	66.60
AMK 100	0.10±0.05*	0.24±0.01*	0.33±0.03*	0.22±0.02	65.62

AMK 300	0.11±0.04*	0.22±0.02*	0.27±0.01*	0.25±0.02*	71.87

n=5. The observations are mean ± SEM. *p < 0.05, compared to control (one-way ANOVA followed by Dunnett's test).

Table 2: Effect of *Murraya koenigii* on SOD, Catalase, GSH & LPO levels in Carrageenan-induced paw edema in rats.

Treatment (mg/kg p.o.)	SOD (U/g wet tissue)	Catalase (U/g wet tissue)	GSH (nmol/mg wet tissue)	LPO (µM/mg wet tissue)
Control	52.07±4.22	3.558±0.20	2.117±0.09	2.27±0.03
Aspirin 100	105.5±2.32*	12.21±0.60*	7.696±0.52*	0.58±0.05*
PMK 100	70.07±1.39*	5.965±0.52*	3.841±0.16*	1.37±0.02*
PMK 300	95.74±7.17*	9.507±0.29*	5.49±0.08*	0.91±0.05*
AMK 100	85.86±4.13*	8.72±0.66*	4.541±0.41*	0.87±0.02*
AMK 300	107±2.20*	11.53±0.21*	6.306±0.29*	0.61±0.03*

n=5. The observations are mean ± SEM. *p< 0.05, compared to control (one-way ANOVA followed by Dunnett's test).

Table 3: Effect of *Murraya koenigii* on the hematological parameters in carrageenan-induced inflammation in rat

Treatment (mg/kg) / **Parameters**	Control	Aspirin 100	PMK 100	PMK 300	AMK 100	AMK 300
Glucose (mg dl^{-1})	116.56 ±2.23	102.53 ±3.26*	112.32 ±2.69*	115.39 ±3.5*	118.23 ±2.5*	109.45 ±1.6*
Hemoglobin (g dl^{-1})	13.93±0.75	6.2±1.59*	11.8±0.68*	12.1± 0.29*	12.23± 0.95*	14.29±1.97*
Red blood cells (Million.m.mm)	4.96±0.02	2.96±0.04*	4.03±0.02*	4.26± 0.1*	4.03±0.09*	4.12±0.08*
Total leukocyte count (cells m^{-1} mm^{-1})	3800±5.4	5300±6.9*	4200±4.32*	4100± 4.8*	3900±5.08*	4500±4.4*
Lymphocyte	26±2.09	46±1.65*	32±1.78*	39±2.65*	38±0.9	42±1.09

					8*	*
Neutrophils	39±1.56	55±2.12*	52±1.89*	63±0.68*	65±1.63*	77±3.36*
Basophils	2.5±0.98	1.12±0.65*	2.69±0.87*	3.12±0.85*	2.79±0.76*	3.42±1.24*
Plateletcount (lakhs.m.mm)	2.97±0.09	1.90±0.15*	2.22±0.55*	2.12±0.87*	2.08±0.98*	1.95±1.6
PCV (%)	44±0.69	28±0.60*	32±0.93*	40±0.5*	39±1.35*	42±4.86*
AST (U/L)	122.32±5.72		75.58±7.2*	62.5±3.65*	66.42±5.78*	55.64±4.90*
ALT (U/L)	129.13±5.34		72.44 ±5.71*	65.29 ±5.32*	62.98 ±5.69*	52.33 ±4.65*

n=5. The observations are mean ± SEM. *p< 0.05, compared to control (one-way ANOVA followed by Dunnett's test).

IV. CONCLUSION

A significant % inhibition of paw edema and alterations in associated biochemical parameters by pet ether extract (PMK) of *Murraya koenigii* leaves and alkaloids (AMK) isolated from PMK at doses of 100 and 300 mg/kg/day, p.o., for 11days, suggest its usefulness as an anti-inflammatory agent.

V. REFERENCES

[1] Aliyu R, Adebayo AH, Gatsing D, Garba IH (2006). The Effects of Ethanolic Leaf Extract of *Commiphora africana* (Burseraceae) on Rat Liver and Kidney Functions. J. Pharmacol. Toxicol. 2: 373-379.

[2] Arulselvan P, Subramanian SP (2007) Beneficial effects of Murraya koenigii leaves on antioxidant defense system and ultra structural changes of pancreatic beta-cells in experimental diabetes in rats" Chem Biol Interact 165 :155–64

[3] Beers R. F. Jr, and Sizer I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J.Biol.Chem. 133:140

[4] Bermond, P. (1989) Analgesic and anti-inflammatory properties of vitamins. Int. J. Vitam. Nutr. Re. 30:153–160

[5] Bhattacharya L, Roy SK and Chakraborty DP (1982). Structure of the carbazole alkaloid isomurrayazoline from murraya koenigii. Phytochemistry. 21: 2432-2433

[6] Boughton-Smith NK, Deakin AM, Follen Fant RL, Whittle BJR, Corlant LG (1999). Role of oxygen radicals and arachidonic acid metabolites in the reserve passive arthus reaction and carrageenan paw edema in rats. British J Pharmacol 110: 896-902

[7] Chakrabarty M, Nath A, Khasnobis S (1997) Carbazole alkaloids from *Murraya koenigii*. Phytochemistry 46 :751-756

[8] Cheesman KH (1993) Lipid peroxidation in the biological systems In: Halliwell B, Arouma OI (Eds.). DNA and free radicals, Ellis Horwood, London, pp: 12-17

[9] Chowdhury BK & Chakraborty DP (1971) Mukeic acid, The first carbazole Carboxylic acid from plant source, Phytochemistry10:1967-1970

[10] Cordell G A (1981) Introduction to the alkaloids: A Biogenetic approach, Wiley Interscience, New York

[11] Da Motta JI, Cunha FQ, Vargafrig BB, Ferreira SH (1994). Drug modulation of antigen induced paw edema in Guinea pig: Effect of lippopolysaccharides, tumor necrosis factor and leucocyte depletion.Br.J. Phamacol 112: 111-116

[12] Das D, Bandyopadhyay D, Bhattacharya M, Banerjee RK (1997) Hydroxyl radical is the major causative factor in stress induced gastric ulceration. Free Radical Biol. Med 23:8-18

[13] Das K, Chakraborty D (1965) Antifungal activity of some constituents of Murraya koenigii sperng. Experentia 21:340

[14] Di Rosa M, Willoughby DA (1971). Screens of anti-inflammatory drugs. J Pharm Pharmacol. 23: 297-298.

[15] Ellman G.L. (1959). Tissue sulfahydryl groups. *Arch. Biochem. Biophys*. 82:70 - 77.

[16] Fernanda LB, Victor AK, Amelia TH (2004) Analgesic property of Umbelletin from Psychotria umbellate., Pharmaceutical Biology 44: 56

[17] FitzGerald G, Patrono C (2001) The coxibs, selective inhibition of cyclooxygenase-2. New Engl J Med 345:433–442

[18] Fridovich I (1986). Biological effects of superoxide radical. Archives Biochem Biophysics 247: 1-11

[19] Gualillo O, Eiras S, Lago F, Dieguez C, Casanueva F (2001) Evaluated serum leptin concentrations induced by experimental acute inflammation. J. Ethanopharmacol.75:213-218

[20] Gupta S, George M, Singhal M, Sharma G, Garg V. (2010) Leaves extract of Murraya koinigii Linn. for Anti-inflammatory Analgesic and activity in animal models, Journal of advanced Pharmaceutical Technology & Research 1:68-77

[21] Gupta V, Sharma M, (2010) Protective effect of *murraya koenigii* on lipid peroxide formation in isolated rat liver homogenate. International journal of pharma and bio sciences1:1-6

[22] Halliwell B. (1995) Antioxidant characterization: Methodology and mechanism. Biochem Pharmacol 49: 1341-48

[23] Jain NK, Kulkarni SK, Singh A (2001) Role of cysteinyl leukotrienes in nociceptive and inflammatory condition in experimental animals. European J Pharmacol 415:85-94

[24] Joshi BS, Smut VN and Gawad DH(1970) On the structures of girinimbine, Mahanimbine, isomahanimbine, koenimbidine and murrayacine .tetrahedron. 26: 1475-1482.

[25] Kirtikar K.R, Basu B.D. Indian Medicinal Plants Vol 1, 2nd edition, Bishen Singh Mahendra Pal Singh, India. 1993:472-474

[26] Mc Cord JM, Roy RS (1982) The pathology of superoxide: Roles in inflammation and ischemia. Can J Physiol Pharm 60: 1346-52

[27] Misra HP, Fridovich I (1972). The generation of superoxide radical during the auto-oxidation of haemoglobin .*J.Biol.Chem*.247: 6960-6962

[28] Mitsuda H, Yuasumoto K, Iwami K (1996). Antioxidation action of indole compounds during the autoxidation of linoleic acid. J. Jpn. Soc. Food Nutr. 19: 210-214

[29] Molyneux P.(2004) Use of DPPH to testing antioxidant activity. J Sci Technol 26: 212-219

[30] Muthumani P, Venkatraman S (2009) Pharmacological studies of anticancer, anti inflammatory activities of *Murraya koenigii* (Linn) Spreng in experimental animals. J. Pharm. Sci. & Res 1: 137-141

[31] Narasimhan NS, Paradhar MV and Chitguppi VP (1968) Structure of Mahanimbin and Koenimbin. Tetrahedron Lett. 53: 5501-5504

[32] Niehaus WG, Samuelsson B (1968) Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem. 6: 126-130

[33] Onasanwo SA, Elegbe RA (2000) Antinociceptive and anti-inflammatory effect of

leaf extract of Hedranthera barter in rats and mice. African J Biomed Res 14: 418

[34] Rao LJM, Ramalakshmi K, Borse BB, Raghavan B(2006) Food Chemistry 100: 742-747

[35] Raybak MEM (1992) Hematologic effects of Nonsteroidal anti-inflammatory drugs. In: Borda IT, Koff RS (eds) NSAIDs a profile of adverse effects, Hanley & Belfus, Philadelphia, pp. 113-32.

[36] Reisch J, Wickramasinghe A, Herath B and Henkel G (1992) Carbazole alkaloids from seeds of murraya koenigii. Phytochemistry 31:2877-2879

[37] Ruch RJ, Cheng SJ and Klauning JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 10: 1003-1008

[38] Saggu H, Cooksey J, and Dexter D. (1989). A selective increase in particulate superoxidase dismutase activity in parkinsonian substantia nigra. *J. Nerochem*. 53:692-697

[39] Scheiman J M and Hindley CE (2010) Strategies to optimize treatment with NSAIDS in patients at risk for gastrointestinal and cardiovascular adverse events. Clinical Therapeutics 32:667-677

[40] Serhan C N and B. Levy (2003) Success of prostaglandin E2 in structure–function is a challenge for structure-based therapeutics. Proc. Natl. Acad. Sci. 100:8609–8611.

[41] Syahida A, Israf DA, Lajis NH, Khozirah S, Habsah M, Permana D, Norhadiani I (2006) Effect of compounds isolated from natural products on IFN-c/LPS-induced nitric oxide production in RAW 264.7 macrophages. Pharm Biol 44:50–59

[42] Trease G E, Evans W C (Eds) (1996) Pharmacognosy. 14th ed. Hawoust Brace and company, London.pp 293

[43] Trenam CW, Blake DR, Morris CJ. (1992) Skin Inflammation: Reactive oxygen species and the role of iron. J Invest Dermatol 99: 675.

[44] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem-Biol Interact 160:1–40

[45] Vogel HG, Vogel Wolfgang H (2002) Drug discovery and evaluation of pharmacological assay. In: 2nd Edn. Springer New York, Springer; pp. 670

[46] Winter CA, Risley EA, Nuss GW(1962) Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol 111: 544-547.

[47] Yakubu MT, Akanji MA, Oladiji AT (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia argrestis* stem. Res. J. Med. Plant. 2: 66-73.