Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (7): 521-528, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.521.528

Analgesic and Anti-inflammatory Activity of Ethanolic Extract of *Zizyphus nummularia*

[1]Manoj Goyal, [2]D. Sasmal and [1]B.P. Nagori
[1]Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, 342008, Rajasthan, India
[2]Department of Pharmaceutical Sciences, BIT Mesra, Ranchi, Jharkhand, India

Corresponding Author: Manoj Goyal, Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, 342008, Rajasthan, India

ABSTRACT

Present study was undertaken to examine anti-inflammatory and analgesic activity of ethanolic extract of *Zizyphus nummularia* (EAZN). EAZN produced anti-inflammatory activity against acute paw oedema induced by carrageenan and histamine at the dose levels of 200 and 300 mg kg^{-1}, at the same dose levels EAZN inhibited peritoneal leukocyte migration. Significant decrease in number of writhes and increase in tail flick latency was observed in acetic acid induced writhing and tail flick test, respectively. In conclusion, it is suggested that inhibition of pain and inflammation mediators is the possible mechanism of action.

Key words: Paw oedema, analgesic, anti-inflammatory

INTRODUCTION

Zizyphus nummularia (family: Rhamnaceae) is a thorny small bush or shrub, grows in abundance in the grazing lands of the arid and semi-arid areas of India. In Rajasthan it forms 14% of the total composition of the grassland flora. It is frequently cultivated food and fodder (Anonymous, 1989).

Tasty sweet and sour fruits of *Zizyphus nummularia* are consumed by all sections of society for its nutritional and medicinal value; young leaves are cooked as vegetable and used as medicine.

Zizyphus nummularia is used by the local community as analgesic, anti-inflammatory, anti-colds and anti-coughs medicine (Anonymous, 1989; Shah *et al*., 1990; Goyal *et al*., 2011; Chanda *et al*., 2011). *Zizyphus nummularia* found to contains many bioactive phytochemical constituents such as pectin, saponins, triterpenoic acids, fatty acids and cyclopeptide alkaloids.

Cyclopeptide alkaloids have been reported for sedative, antimicrobial, hypoglycemic, antiplasmodial, anti-infectious, antidiabetic, diuretic, anticonvulsant, analgesic and anti-inflammatory activities.

The extractive value of ethanolic extract of *Zizyphus nummularia* is found to be high and alkaloids present in *Zizyphus* can be extracted with alcohol (Morel *et al*., 2009; Ma *et al*., 2008). Present study was undertaken to examine analgesic and anti-inflammatory effects of ethanolic extract of leaves of *Zizyphus nummularia* to validate ethnomedicinal claims.

MATERIALS AND METHODS

Plant material: *Zizyphus nummularia* is commonly grown in Rajasthan. The leaves were collected in March 2008 from Jodhpur. Herbarium of plant was identified by

Taxonomist of Botanical Survey of India (BSI), Jodhpur, Rajasthan. A voucher specimen was deposited in the BSI.

Preparation ethanolic extract: One kilogram of dried and powdered leaves was extracted three times in a reflux condenser for 24 h with hexane. The residues were extracted with ethanol for 48 h and the ethanolic extract was evaporated to dryness by rotary evaporation. The yield of ethanolic extracts of *Zizyphus nummularia* was about 12% (Ma *et al*., 2008).

Chemicals and drugs: Carrageenan (S.D. Fine Chemicals Limited, Bombay), histamine (Sigma, USA), indomethacin (Recon, Bangalore), aspirin (USV Bombay), morphine (Bio E,) were used in the study.

Animal material: Male Wistar rats weighing 180-200 g and male Swiss mice weighing 20-24 g were used. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at 25°C with a 12 h light/dark cycle. The experiments were performed during the light portion (0800-1600 h). The experimental protocol and procedures used in this study were approved by IAEC of Lachoo Memorial College of Science and Technology, Pharmacy Wing, Jodhpur, Rajasthan.

Anti-inflammatory activity

Carrageenan-induced rat paw oedema: The rats were divided into five groups (n = 6). The different groups were treated with ethanolic extract of leaves of *Zizyphus nummularia* (EAZN) (100, 200 and 300 mg kg^{-1} b.wt., p.o.), indomethacin (10 mg kg^{-1} b.wt., p.o.) and vehicle control (5 mL kg^{-1} b.wt., p.o.). The animals were treated with the EAZN 1 h before the administration of carrageenan. Acute inflammation was produced by the subplantar administration of 0.1 mL of 1% carrageenan in normal saline in the right hind paw of the rats. The paw volume was measured at 0 and 3 h after carrageenan injection using plethysmometer (Winter and Porter, 1957). The anti-inflammatory effect of EAZN was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}D/C)\times 100$$

where, D represents the percentage difference of paw volume of EAZN treated groups and C represents the percentage difference of volume in the control group (Suleyman *et al*., 1999; Aderogba *et al*., 2006; Awe *et al*., 2006).

Histamine-induced inflammation: The anti-inflammatory activity of the EAZN was measured against histamine which act as mediator of inflammation (Winter *et al*., 1962). Paw oedema was induced in rats by subplantar injection of 0.1 mL of freshly prepared histamine (1 mg kg^{-1} b.wt.) solutions.

Mouse carrageenan peritonitis: The mice were divided into five groups (n = 6). The EAZN (100, 200 and 300 mg kg^{-1} b.wt., p.o.), indomethacin (10 mg kg^{-1} b.wt., p.o.) and vehicle control (5 mL kg^{-1} b.wt., p.o.) were administered. One hour later peritonitis was induced by intraperitoneal

injection of carrageenan (0.25 mL, 0.75% in saline). After 4 h the animals were sacrificed by high dose of anesthesia and peritoneal fluid collected for total and differential leukocyte count (Griswold *et al*., 1987). Percentage inhibition was calculated by the following equation:

$$\text{Percentage inhibition} = (1\text{-}D/C)\times 100$$

where, D represents the leukocyte counts of treated groups and C represents the leukocyte counts of the control group.

Analgesic activity

Acetic acid-induced writhing response in mice: Five groups of mice were selected for the study (n = 6). Group one received saline (5 mL kg^{-1} b.wt., p.o.), group two received aspirin (100 mg kg^{-1} b.wt., p.o.) and remaining three groups of mice received EAZN at the dose of 100, 200 and 300 mg kg^{-1} b.wt., p.o. (Gupta *et al*., 2005). Thirty minuets after drug treatment, 0.1 mL of 0.6% solution of acetic acid was injected intraperitoneally and the number of writhes during the following 30 min period were counted (Koster *et al*., 1959; Shilpi *et al*., 2005; Usman *et al*., 2008; Arora *et al*., 2011; Shehab *et al*., 2011). Percentage inhibition was calculated by the following equation:

$$\text{Percentage inhibition} = (1\text{-}D/C)\times 100$$

where, D represents the number of writhes of treated groups and C represents the number of writhes of the control group.

Tail flick reaction time in mice: Five groups of mice were selected for the study (n = 6), mice which had 3.5-4.5 seconds baseline latency of tail flick were included in study. Group one received saline (5 mL kg^{-1} b.wt., p.o.), group two received morphine (5 mg kg^{-1} b.wt., s.c. injection), remaining three groups of mice received 100, 200 and 300 mg kg^{-1} b.wt., p.o. of EAZN. Morphine and EAZN were given 30 and 60 min before the test.

Mice were screened by placing their proximal third of the tail to radiant heat source maintained at 50±2°C, latency for tail flick was recorded. A cutoff time of 20 sec was used to avoid damage to the tail (D'Amour and Smith, 1941; Al-Howiriny, 2004; Chowdhury *et al*., 2005; Rakhshandeh *et al*., 2008; Gill *et al*., 2011). The Percentage anti-nociceptive activity was calculated following equation:

$$\text{Percentage anti-nociceptive activity} = (1\text{-}D/C)\times 100$$

where, D represents the latency of tail flick of treated groups and C represents the latency of tail flick of the control group.

Acute toxicity: For the acute toxicity assay, two groups of three male mice (20-24 g) were made. The animals were kept without access to food and water. The assay was followed as OECD Guideline 423 (OECD, 2001). The control group received normal saline 1 mL kg^{-1} by gavage while the exposed group received 1000 mg kg^{-1} of EAZN. The safety of 1000 mg kg^{-1} dose was

subsequently confirmed in another three animals as recommended in the OECD guideline. Immediately after dosing, the animals were observed continuously for symptoms of toxicity for 4 h in terms of autonomic and neurobehavioral alterations. They were then kept under observation up to 14 days in terms of weight loss and chow consumption. On day 15, the animals were euthanized and their vital organs were individually observed for overt pathology.

Statistical analysis: Values were expressed as Mean±SEM, statistical significance was determined by one way ANOVA followed multiple comparisons versus control group by Dunnett's Method; values with $p<0.05$ were considered as statistically significant.

RESULTS

Rat paw oedema: The EAZN produced anti-inflammatory activity against acute paw oedema induced by carrageenan and histamine (Table 1). The anti-inflammatory effect found to be statistically significant ($p<0.05$) only at the dose levels of 200 and 300 mg kg^{-1}.

Mouse carrageenan peritonitis: EAZN at the dose levels of 200 and 300 mg kg^{-1} inhibited peritoneal leukocyte migration (Table 2), inhibition is found to be statistically significant ($p<0.05$) and dose dependent.

Acetic acid-induced writhing in mice: Significant ($p<0.05$) decrease in number of writhes was observed (Fig. 1). at dose levels 200 and 300 mg kg^{-1}. In test group maximum inhibitions of writhes was observed in group treated with 300 mg kg^{-1} EAZN (Fig. 2).

Table 1: Effect of Ethanolic extract of *Zizyphus nummularia* and indomethacin on carrageenan and histamine-induced pedal oedema in rats

		Increase in paw oedema (Mean+SEM) and % inhibition of paw oedema			
		Carrageenan		Histamine	
Treatment	Dose mg kg^{-1} (p.o.)	Paw oedema	Inhibition (%)	Paw oedema	Inhibition (%)
Control	5 (mL kg^{-1})	0.86±0.02		0.95±0.03	
Indomethacin	10	0.32±0.01*	62.79	0.26±0.02*	72.63
EAZN	100	0.85±0.02	01.16	0.92±0.03	03.15
EAZN	200	0.62±0.05*	27.90	0.68±0.04*	28.42
EAZN	300	0.53±0.02*	38.37	0.55±0.02*	42.10

Values are expressed as the Mean±SEM of six observations, *$p<0.05$ Statistical comparisons are made between: Control vs. Indomethacin, I, II and group III

Table 2: Effect of Ethanolic extract of *Zizyphus nummularia* and indomethacin on carrageenan induce peritonitis in mice

Treatment	Dose mg kg^{-1} (p.o.)	Leukocytes (10^6 mL)	Leukocytes inhibition
Control	5 (mL kg^{-1})	4.32±0.21	-
Indomethacin	100	2.10±0.12*	51.32
EAZN	200	4.20±0.11	02.77
EAZN	300	3.42±0.14*	20.83
EAZN	100	2.50±0.08*	42.12

Values are expressed as the Mean±SEM of six observations. "*" $p<0.05$ Statistical comparisons are made between: Control versus Indomethacin, I, II and group III

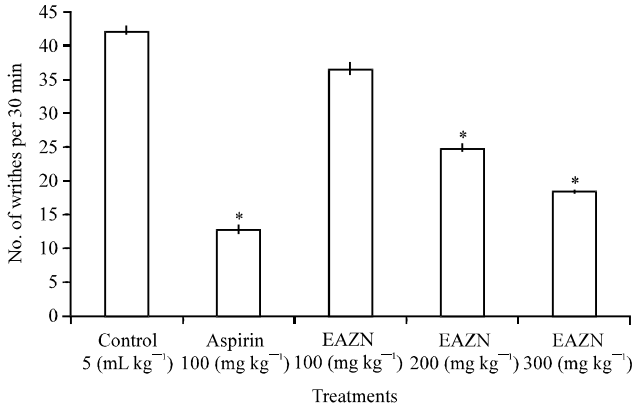

Fig. 1: Effect of Ethanolic extract of *Zizyphus nummularia* and aspirin on acetic acid induce writhing in mice. Values are expressed as the Mean±SEM of six observations, *p<0.001 statistical comparisons are made between: control vs. aspirin, I, II and group III

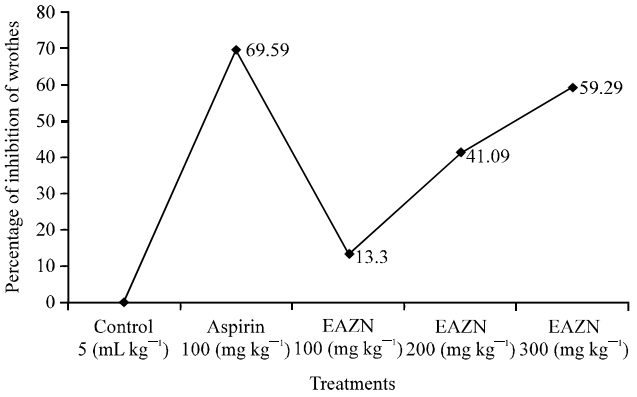

Fig. 2: Percentage inhibitions of writhes by ethanolic extract of *Zizyphus nummularia* and aspirin

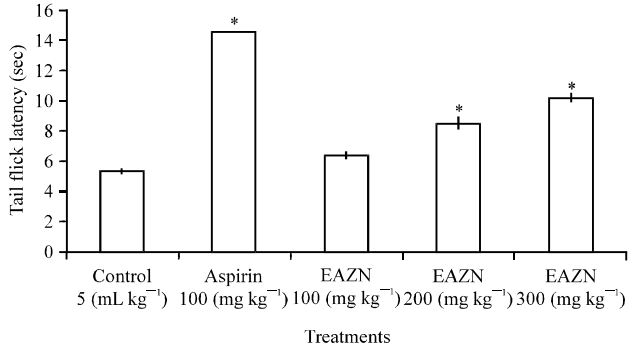

Fig. 3: Effect of ethanolic extract of *Zizyphus nummularia* and morphine on tail flick latency of mice. Values are expressed as the Mean±SEM of six observations, *p<0.001 Statistical comparisons are made between: control vs. morphine, I, II and group III

Tail flick latency in mice: Significant increase in tail flick latency ($p<0.05$) was observed at dose levels of 200 and 300 mg kg^{-1} (Fig. 3). EAZN 300 mg kg^{-1} produces 90% anti-nociceptive activity (Fig. 4).

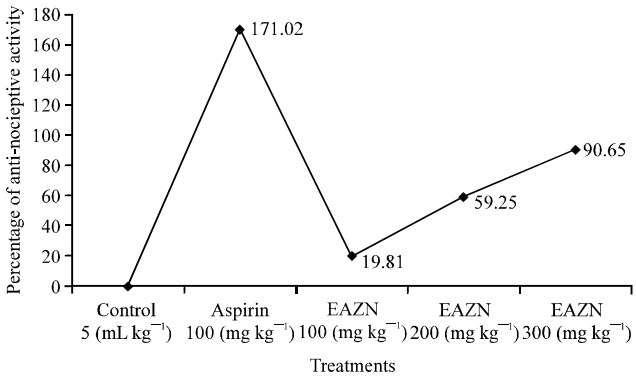

Fig. 4: Percentage anti-nociceptive activity of ethanolic extract of *Zizyphus nummularia* and morphine on tail flick latency of mice

DISCUSSION

This study evaluated the putative analgesic and anti-inflammatory activities of the ethanolic extract of leaves of *Zizyphus nummularia* to validate ethnomedicinal claims made regarding the *Zizyphus nummularia*. Carrageenan-induced paw edema as an *in vivo* model of inflammation has been frequently used to assess the antiedematous effect of natural products. The EAZN showed dose-dependent anti-oedematogenic effects on paw oedema induced by carrageenan. The cellular and molecular mechanism of the carrageenan-induced inflammation well characterized. It is known that carrageenan oedema is mediated through release of inflammation mediators such as serotonin and histamine (Linardi *et al*., 2000). The EAZN causes pronounced reduction in the paw oedema induced by histamine and reduces vascular permeability in carrageenan induced peritonitis, results suggests that anti-inflammatory activity of the EAZN is possibly backed by its anti-histaminic activity. Since histamine is important mediators of inflammation, causes vasodilation and increases the vascular permeability (Cuman *et al*., 2001).

In acetic acid-induced abdominal writhing which is the visceral pain model, the result indicated that all the doses produced significant analgesic effect. This could be attributed, partly, to its anti-inflammatory effect as, in the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism (Franzotti *et al*., 2000). Thus, the results obtained for the writhing test are similar to those obtained for the oedematogenic test using carrageenan. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity because inhibition of the acute inflammation by this extract led to their inhibitory effect on pain development.

Tail-flick test is used for screening of centrally acting analgesics, tail flick to noxious thermal stimuli are mediated via supra-spinal centers (Dewey *et al*., 1970; Kazunaga *et al*., 1980). EAZN was found to be effective in tail flick test in mice which indicate the analgesic activity by central mechanism.

Cyclopeptide alkaloid faction of Zizyphi Spinosi Semenis found to enhance pentobarbital-induced sleeping behaviors and this action is mediated through GABA receptors Cl^- channel activation. In addition, Cyclopeptide alkaloid in combination with $GABA_A$ receptors agonist muscimol, synergistically prolonged pentobarbital-induced sleeping time (Ma *et al*., 2008). Muscimol also causes latency in tail flick, so, another possibility of positive results of tail flick is GABA agnostic action (Aanonsen and Wilcox, 1989).

CONCLUSION

In conclusion, since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine, as well as reduced the number of writhes in acetic acid-induced writhing models and increases tail flick latency, the EAZN exhibited anti-inflammatory and analgesic activities. The study has thus provided some justification for the folkloric use of the plant in several communities for conditions such as pain and inflammations.

REFERENCES

Aanonsen, L.M. and G.L. Wilcox, 1989. Muscimol, gamma-aminobutyric acid: A receptors and excitatory amino acids in the mouse spinal cord. J. Pharmacol. Exp. Ther., 248: 1034-1038.

Aderogba, M.A., E.K. Okoh, I.N. Okeke, A.O. Olajide and A.O. Ogundaini, 2006. Antimicrobial and anti-inflammatory effects of *Piliostigma reticulatum* leaf extract. Int. J. Pharmacol., 2: 70-74.

Al-Howiriny, T.A., M.A. Al-Yahya, M.S. Al-Said, K.E.H. El-Tahir and S. Rafatullah, 2004. Studies on the pharmacological activities of an ethanol extract of balessan (*Commiphora opobalsamum*). Pak. J. Biol. Sci., 7: 1933-1936.

Anonymous, 1989. The Wealth of India (Raw Material). Council of Industrial and Scientific Research, New Delhi, Pages: 590.

Arora, R., M. Kaur and N.S. Gill, 2011. Antioxidant activity and pharmacological evaluation of *Cucumis melo* var. *agrestis* methanolic seed extract. Res. J. Phytochem., 5: 146-155.

Awe, E.O., J.M. Makinde, O.A. Olajide and O.K. Wakeel, 2006. Membrane stabilizing activity: A possible mechanism of action for the anti-inflammatory and analgesic properties of *Russelia equisetiformis*. Int. J. Pharmacol., 2: 447-450.

Chanda, S., R. Dave and M. Kaneria, 2011. *In vitro* antioxidant property of some Indian medicinal plants. Res. J. Med. Plant, 5: 169-179.

Chowdhury, K.K., A. Saha, S.C. Bachar and J.K. Kundu, 2005. Analgesic and anti-inflammatory activities of *Desmodium triflorum* DC. J. Biological Sci., 5: 581-583.

Cuman, R.K.N., C.A. Bersani-Amado and Z.B. Fortes, 2001. Influence of type 2 diabetes on the inflammatory response in rats. Inflamm. Res., 50: 460-465.

D'Amour, F.E. and D.L. Smith, 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Therapeut., 72: 74-79.

Dewey, W.L., L.S. Harris, J.F. Howes and J.A. Nuite, 1970. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J. Pharmacol. Exp. Ther., 175: 435-442.

Franzotti, E.M., C.V.F. Santos, H.M.S.L. Rodrigues, R.H.V. Mourao, M.R. Andrade and A.R. Antoniolli, 2000. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). J. Ethnopharmacol., 72: 273-277.

Gill, N.S., R. Arora and S.R. Kumar, 2011. Evaluation of antioxidant, anti-inflammatory and analgesic potential of the *Luffa acutangula* Roxb. var. *amara*. Res. J. Phytochem., 5: 201-208.

Goyal, M., D. Sasmal and B.P. Nagori, 2011. Review on medicinal plants used by local community of Jodhpur district of Thar Desert. Int. J. Pharmacol., 7: 333-339.

Griswold, D.E., P.J. Mrarshall, E.F. Webb, R. Godfrey and J. Newton *et al.*, 1987. Inhibition of cytokine production as well as both fatty acid. Biochem. Pharmacol., 36: 3463-3470.

Gupta, M., U.K. Mazumder, R.S. Kumar, P. Gomathi, Y. Rajeshwar, B.B. Kakoti and V.T. Selven, 2005. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhinia racemosa* stem bark in animal models. J. Ethnopharmacol., 98: 267-273.

Kazunaga, F., K. Osamu, H. Morihide, M. Noriyuki, O. Seiichi and H. Yoshikazu, 1980. A method for evaluating analgesic agents in rats. J. Pharm. Methods, 4: 251-259.

Koster, R., M. Anderson and E.J. De Beer, 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412-418.

Linardi, A., S.K.P. Costa, G.R. da Silva and E. Antunes, 2000. Involvement of kinins, mast cells and sensory neurons in the plasma exudation and paw oedema induced by staphylococcal enterotoxin B in the mouse. Eur. J. Pharmacol., 399: 235-242.

Ma, Y., H. Han, S.Y. Nam, Y.B. Kim, J.T. Hong, Y.P. Yun and K.W. Oh, 2008. Cyclopeptide alkaloid fraction from *Zizyphi spinosis* semen enhances pentobarbital-induced sleeping behaviors. J. Ethnopharmacol., 117: 318-324.

Morel, A.F., G. Maldaner and V. Ilha, 2009. Alkaloids: Chemistry and Biology. Elsevier, UK., pp: 79-141.

OECD, 2001. Guidelines for testing of chemical. Guideline 423, Acute Oral Toxicity-Acute Toxic Class Method. OECD, Paris.

Rakhshandeh, H., N. Vahdati-mashhadian, K. Dolati and M. Hosseini, 2008. Antinociceptive effect of *Rosa damascena* in Mice. J. Biol. Sci., 8: 176-180.

Shah, A.H., M. Tariq and M.A. Al-Yahyam, 1990. Studies on the alkaloidal fraction from the stem bark of *Zizyphus nummularia*. Fitoterapia, 61: 452-469.

Shehab, N.G., A. Mahdy, S.A. Khan and S.M. Noureddin, 2011. Chemical constituents and biological activities of *Fagonia indica* Burm F. Res. J. Med. Plant., 5: 531-546.

Shilpi, J.A., R. Rouf, M.A.M. Sarker, Qamrunnahar, M.M. Ferdous and S.J. Uddin, 2005. Antinociceptive activity of methanol extract of *Solanum sisymbrifolium* Lamk. Pak. J. Biol. Sci., 8: 1123-1125.

Suleyman, H., L.O. Demirezer, A. Kuruuzum, Z.N. Banoglu, F. Gocer, G. Ozbakir and A. Gepdiremen, 1999. Anti-inflammatory effect of the aqueous extract from *Remex patientia* L. roots. J. Ethnopharmacol., 65: 141-148.

Usman, H., A.H. Yaro and M.M. Garba, 2008. Analgesic and anti-inflammatory screening of *Newbouldia laevis* flower in rodents. Trends Med. Res., 3: 10-15.

Winter, C.A. and C.C. Porter, 1957. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities in hydrocortisone ester. J. Am. Pharma. Assoc., 46: 515-519.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carrageenan induced oedema in hind paw of the rats as an assay of anti-inflammatory drug. Proc. Soc. Exp. Biol. Med., 111: 544-547.