Int. J. Chem. Sci.: 13(4), 2015, 1794-1804
ISSN 0972-768X
www.sadgurupublications.com

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF 3,3-DIMETHYL 2,6- DIMETHYL PIPERIDINE 4-ONE OXIME

K. THARINI[*] and P. SANGEETHA[a]

Department of Chemistry, Govt. Arts College, TIRUCHIRAPPALLI (T.N.) INDIA
[a]Department of Chemistry, Rajah Serfoji Govt. College, THANJAVUR (T.N.) INDIA

ABSTRACT

Antioxidant and anti-inflammatory activity of 1,3-dimethyl 2,6-diphenyl piperidine 4-one oxime were investigated. *In vitro* antioxidant activity like DPPH and superoxide radical scavenging activity were investigated by standard methods. Results of the present study indicates that higher dose of 3, 3-dimethyl 2,6-dimethyl piperidine 4-one oxime has potent anti-inflammatory activity close to standard drug. The above results confirmed that 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime possess significant anti-inflammatory activity as compared to standard drug dexamethasone. The antioxidant activity of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime was concentration dependent and approximately comparable to commercial synthetic antioxidants as ascorbic acid. Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema. After 12 hrs fast rats, were divided into five groups of six each. Each animal was marked for identification and regularly monitoring. Group I served as control group received carrageenan only. Group II, III and IV animals received 3,3-dimethyl 2,6-dimethyl piperidine 4-one oximeat a dose of 100, 250 and 500 mg/Kg orally. Group V was orally administered 2 mg/Kg (ip) dexamethasone as a standard drug. On the basis of the results, it is clearly indicated that 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime had powerful anti-inflammatory and *in vitro* antioxidant activity.

Key words: 1,3-Dimethyl 2,6-diphenyl piperidine 4-one oxime, *In vitro* antioxidant, Anti-inflammation.

INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair[1].

It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation

[*]Author for correspondence; E-mail: tharinilenin@gmail.com

and membrane alterations[2]. Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue[3]. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis[4]. In appreciating the inflammatory process, it is important to understand the role of chemical mediators. There are substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/ macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short-lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin-1)[5].

It is believed that current drugs available such as opoids and non-steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side effects and potency[6]. As a result, a search for other alternatives seems necessary and beneficial. Therefore, there is a need for new and safe anti-inflammatory agents. Research in the recent past has accumulated enormous evidences revealing that enrichment of body systems with antioxidants may correct the vitiated homeostasis and can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxidative stress. These developments accelerated the search for antioxidant principles that lead the identification of natural resources, isolation of active principles and further modification and refinement of active antioxidant molecules[7]. Therefore, the screening and development of drugs for their anti-inflammatory and antioxidant activity is the needed and there are many efforts for finding the anti-inflammatory and antioxidant drugs from synthetic compounds. Keeping in view, in the present study, the anti-inflammatory and antioxidant activity of 3,3-dimethyl 2,6-diphenyl piperidine 4-one oxime have been investigated.

EXPERIMENTAL

Materials and methods

Preparation of 3, 3-dimethyl 2,6-dimethyl piperidine 4-one

Ammonium acetate (100 mL), benzaldehyde (200 m mol) and corresponding ketone (100 m mol) in the molar ratio of 1:2:1 were added to ethanol (70 mL) and the mixture was heated untill the colour of the solution changed to yellow. The solution was kept aside at

room temperature for crystallization. The solid precipitated was filtered, washed with ether and ethanol mixture (90:10% v/v) and recrystallised from ethanol. Observed melting point was 113°C.

Preparation of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime

The oximes was prepared from the respective ketones by the following procedure **(Scheme 1)**. Hydroxylamine hydrochloride (40 m mol) and sodium acetate (80 m mol) were dissolved in ethanol (50 mL) and the NaCl formed was filtered off. The filtrate was added to the solution of corresponding piperidine 4-one (20 m mol) in ethanol (100 mL) and the mixture was heated under reflux for 4 hrs. The reaction mixture was concentrated and then poured into water (300 mL). The solid obtained was filtered, washed with water and recrystallised from ethanol. Observed melting point was 188 °C.

Experimental animal

Male albino rats of Wistar strain approximately weighing 180-190 g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2ºC and 12 hr light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema according to the technique of Winter et al.[8] After 12 hrs fast, rats were divided into five groups of six each. Each animal was marked for identification and regularly monitoring. Group I served as control group received carrageenan only. Group II, III and IV animals received 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime at a dose of 100, 250 and 500 mg/kg orally. Group V was orally administered 2 mg/kg (ip) dexamethasone as a standard drug. The animals were pretreated with the 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime half an hour 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 paw of the control and experimental rats. The paw was marked with in at the level of lateral malleous and immersed in mercury up to the mark and measured by mercury volume displacement methods. The paw volume was measured ½, 1, 1½ 2 and 2½ hrs after

injection of carrageenan to each group. The difference between the readings was taken as the volume of oedema and the percentage of anti-inflammatory activity was calculated[8,9].

$$\% \text{ of inhibition rate} = \frac{V_c - V_t}{V_c} \times 100 \qquad \ldots(1)$$

Where V_c is the oedema value of the control group and V_t is the oedema value of treated groups.

In vitro antioxidant activity

Different concentrations of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime (20, 40, 60 and 80 µg/mL) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid (20, 40, 60 and 80 µg/mL) was used as a standard.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined by the method of Shimada et al.[10] Briefly, a 2 mL aliquot of DPPH methanol solution (25 µg/mL) was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C}\right) \times 100 \qquad \ldots(2)$$

Where A_C = Control is the absorbance and A_S = Sample is the absorbance of reaction mixture (in the presence of sample).

Superoxide anion scavenging activity assay

The superoxide anion radicals scavenging activity was measured by the method of Liu and Chang[11]. In these experiments, the superoxide anion was generated in 3 mL of tris-HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 µM) solution, 0.75 mL of NADH (936 µM) solution and 0.3 mL of different concentrations of the sample. The reaction was initiated by adding 0.75 mL of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100) \qquad \ldots(3)$$

Where A_0 was the absorbance of the control (blank, without sample) and A_1 was the absorbance in the presence of the sample.

Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a non-linear regression algorithm.

RESULTS AND DISCUSSION

Anti-inflammatory

Carrageenan induced inflammation is a biphasic phenomenon and is a useful model to detect oral actions of anti-inflammatory agents[12]. The development of oedema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances[8]. Inflammation is clinically defined as a pathophysiological process characterized by redness, edema, fever, pain, and loss of function. Although the currently used steroidal anti-inflammatory drugs (SAID) and non-steroidal anti-inflammatory drugs (NSAID) treat acute inflammatory disorders, these conventional drugs have not been successful to cure chronic inflammatory disorders such as rheumatoid arthritis (RA) and atopic dermatitis (AD). Since the critical etiology and exacerbating mechanisms are not completely understood, it is difficult to develop a magic bullet for chronic inflammatory disorders. Therefore, there is a need for new and safe anti-inflammatory agents and one of the ongoing researches in pharmaceutical industry[13].

The effect of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oximeon carrageenan induced paw oedema was calculated, and the result was presented in Table 1. The rat's foot pads become edematous after injection of carrageenan. Administration of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime reduces the paw oedema to inflammatory rats at a dose of 100, 200 and 500 mg (Kg body weight). The dose dependent significant decrease of paw oedema and the reference drug dexamethasone (2 mg/Kg body weight) exhibited significant decrease. Among the various doses, the 500 mg/Kg (body weight) of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime possess potential anti-inflammatory activity as compared to other doses.

Histamine is one of the important inflammation mediators and it is a potent vasodilator substance and increases the vascular permeability[14]. This study showed that all

the doses of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime effectively suppressed the oedema produced by histamine, so it may be suggested that its anti-inflammatory activity is possibly backed by its antihistaminic activity. The significant activities of the standard drug were also observed. The 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime also effectively suppressed the inflammation produced by serotonin induced by hind paw edema, which indicates that the 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime may exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandins that might be involved in inflammation. From the above results it is suggested that the anti-oedematogenic effects of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime on carrageenan mediators-induced paw oedema may be related to inhibition of inflammation mediator formation.

Table 1: Effect of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime on carrageenan induced paw oedema

Treatment groups	½ hr	1 hr	1 ½ hrs	2 hrs	2 ½ hrs
Group I (Control) (%)	-	-	-	-	-
Group II (%) (100 mg/mL)	13.31 ± 0.93	26.01 ± 3.57	53.81 ± 4.46	66.23 ± 4.63	71.33 ± 4.99
Group III (%) (200 mg/mL)	29.73 ± 2.78	48.73 ± 4.39	57.31 ± 4.64	68.97 ± 5.10	76.70 ± 5.08
Group IV (%) (500 mg/mL)	33.21 ± 2.32	53.51 ± 4.44	64.42 ± 4.50	72.57 ± 4.72	80.24 ± 5.12
Group V (%) (Standard)	31.90 ± 2.23	54.39 ± 3.80	66.06 ± 4.62	67.78 ± 4.74	78.31 ± 5.48

Values were expressed as mean ± SD I six rats in each group

In vitro antioxidant activity

The antioxidant is any substance, which when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of substrate. The term oxidizable substrate includes almost everything found in the living cells including proteins, lipids, DNA and carbohydrates[15]. Biological antioxidants have been defined as compounds that protect biological systems against the potentially harmful effects

of processes or reaction that can cause excessive oxidation. Low levels of one or more of the essential antioxidants have been shown to be associated with many disorders including cancer, inflammation, atherosclerosis, coronary heart disease and diabetes. Thus, in such cases, the administration of exogenous antioxidants seems to be salutary. Nowadays, a great deal of effort being extended to find effective antioxidants for the treatment or prevention of free radical-mediated deleterious effects[16].

DPPH Assay

Recently, the use of the DPPH• reaction has been widely diffused among researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability[17]. DPPH radical scavenging activity of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime and standard ascorbic acid are presented in Fig. 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants[18]. The half inhibition concentration (IC_{50}) of ascorbic acid and 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime were 35.3 μg mL^{-1} and 57.15 μg mL^{-1}, respectively (Table 2 and Fig. 1). The 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Table 2: DPPH radical scavenging and superoxide radical scavenging activity of 1,3-dimethyl 2, 6-diphenyl piperidine 4-one oxime

Concentrations (μg/mL)	DPPH	Ascorbic acid (Standard)	SOA	Ascorbic acid (Standard)
20	14.63 ± 0.95	25.6 ± 2.04	19.75 ± 1.31	31.25 ± 2.50
40	32.81 ± 2.22	61.26 ± 4.90	41.62 ± 2.84	64.23 ± 5.13
60	51.00 ± 3.5	88.98 ± 7.11	58.37 ± 4.15	89.54 ± 7.16
80	73.76 ± 5.09	99.34 ± 7.94	82.25 ± 5.68	98.51 ± 7.88
IC_{50} (μg/mL)	57.15	35.03	48.41	31.62

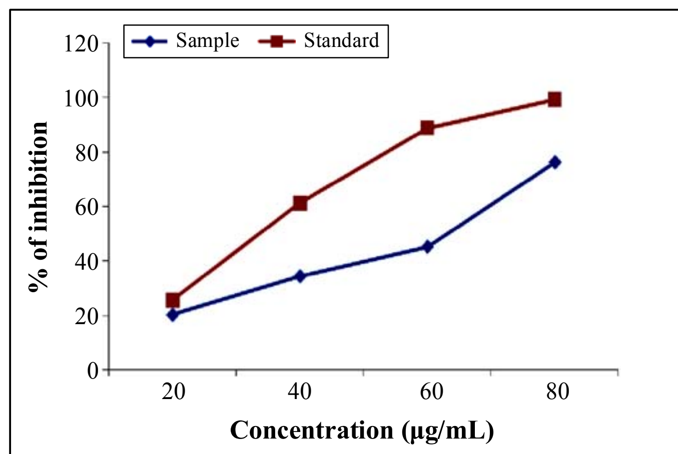

Fig. 1: DPPH radical scavenging activity of 1,3-dimethyl 2,6-diphenyl piperidine 4-one oxime

Superoxide anion radical scavenging activity

Superoxide is biologically important, since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system[19]. The superoxide anion radical scavenging activities of the 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime assayed by the PMS-NADH system were shown in Fig. 2.

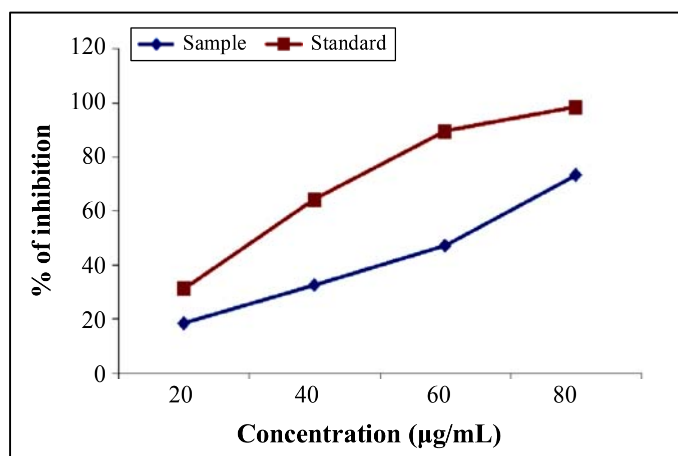

Fig. 2: Superoxide radical scavenging activity of 1,3-dimethyl 2,6-diphenyl piperidine 4-one oxime

The superoxide scavenging activity of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime was 48.41 μg mL^{-1} and ascorbic acid were 31.62 μg mL^{-1} respectively (Table 2 and Fig. 2). These results suggested that 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime had notably superior superoxide radical scavenging effects.

CONCLUSION

In vitro antioxidant and anti-inflammatory activity of 3,3-dimethyl 2, 6-dimethyl piperidine 4-one oxime were tested. Results of the present study indicates that higher dose of 3, 3-dimethyl 2,6-dimethyl piperidine 4-one oxime has potent anti-inflammatory activity close to standard drug. The above results confirmed that 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime possess significant anti-inflammatory activity as compared to standard drug dexamethasone. The antioxidant activity of 3, 3-dimethyl 2,6-dimethyl piperidine 4-one oxime was concentration dependent and approximately comparable to commercial synthetic antioxidants as ascorbic acid. On the basis of the results of this study, it clearly indicates that 3,3-dimethyl 2,6-dimethyl piperidine 4-one oxime had powerful *in vitro* antioxidant and anti-inflammatory activity.

REFERENCES

1. J. R. Vane and R. M Botting, New Insights into the Mode of Action of Anti-Inflammatory Drugs, Inflammation Res., **44(1)**, 1-10 (1995).
2. E. Umapathy, E. J. Ndebia, A. Meeme, B. Adam, P. Menziura, B. N. Nkeh-Chungag and J. E. Iputo, An Experimental Evaluation of Albuca setosa Aqueous Extract on Membrane Stabilization, Protein Denaturation and White Blood Cell Migration During Acute Inflammation, J. Med. Plant Res., **4(5)**, 789-795 (2010).
3. C. W Denko, A Role of Neuropeptides in Inflammation, In: J. T. Whicher, S. W. Evans, Eds. Biochemistry in Inflammation, Kluwer Publisher, London (1992) pp. 177-181.
4. P. M. Henson and R. C. Murphy, Mediators of the Inflammatory Process, Elsevier, Amsterdam (1989) p. 404.
5. G. R Smith and S. Missailidis, Cancer, Inflammation and the AT 1 and AT 2 Receptors, J. Inflammation, **1(3)** , doi:10.1186/1476-9255-1-3 (2004).

6. A. Ahmadiani, M. Fereidoni, S. Semnanian, M. Kamalinejad and S. Saremi, Antinociceptive and Anti-inflammatory Effects of Sambucus Ebulus Rhizome Extract in Rats, J. Ethnopharmacol., **61(2)**, 229-232 (1998).

7. S. Velavan, Free Radicals in Health and Diseases- A Mini Review, Pharmacology Online Newsletter, **1**, 1062-1077 (2011).

8. C. A Winter, E. A Risley and G. W Nuss, Carrageenan-induced Oedema in Hind Paw of the Rats as Assay for Anti-inflammatory Drugs, Proc. Soc. Expe. Biol. Med., **11(1)**, 544- 547 (1962).

9. M. N Ghosh, Fundamentals of Experimental Pharmacology, Hilton & Co., Kolkata (2008).

10. K. Shimada, K. Fujikawa, K. Yahara and T. Nakamura, Antioxidative Properties of Xanthum on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion, J. Agr. Food Chem., **40**, 945-948 (1992).

11. F. Liu and S. T. Chang, Free Radical Scavenging Activity of Mushroom Polysaccharide Extracts, Life Sci., **60**, 763-771 (1997).

12. R. Meier, W. Schuler and P. Desaulles, Leusic Acid, Tumor Inhibitor Isolated from Lichens, Experimentation, **6**, 469-471 (1950).

13. N. Shan Biren, B. S. Nayak and A. K. Seth, Search of Medicinal Plants as a Source of Anti-inflammatory and Anti-arthritic Agents, Pharmacogony Mag., **2**, 77-80 (2006).

14. G. Higgs and T. Willam, Inflammatory Mediators, CRC Press, New York (1985).

15. N. I. Krinsky, Mechanism of Action Biological Antioxidants, Proc. Soc. Exp. Biol. Med., **200**, 248-254 (1992).

16. R. J. Reiter and G. D. Robinson, Where Do Free Radicals Come From? Melatonin, Bantam Book, USA (1995) p. 24.

17. M. Sindhu and T. E Abraham, *In Vitro* Antioxidant Activity and Scavenging Effects of Cinnamomum Verum Leaf Extract Assayed by Different Methodologies, Food Chem. Toxicol., **44**, 198-206 (2006).

18. A. M. Nuutila, R. P. Pimia, M. Aarni and K. M. O. Caldenty, Comparision of Antioxidant Activities of Onion and Garlic Extracts by Inhibition of Lipid Peroxidation and Radical Scavenging Activity, Food Chem., **81**, 485-493 (2003).

19. M. Korycka-Dahl and M. Richardson, Photogeneration of Superoxide Anion in Serum of Bovine Milk and in Model Systems Containing Riboflavin and Aminoacids, J. Dairy Sci., **61**, 400-407 (1978).

Revised : 22.10.2015 *Accepted : 24.10.2015*