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ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF 1,3-DIMETHYL 2,6-DIPHENYL PIPERIDINE 4-ONE OXIME

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ABSTRACT

To investigate the anti-inflammatory and antioxidant activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime. Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema. After 12hrs 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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime at a dose of 100, 250 and 500 mg/kg orally. Group V was orally administered 2mg/kg (ip) Dexamethasone as a standard drug. *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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime has potent anti-inflammatory activity close to standard drug. The above results confirmed that 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime possess significant anti-inflammatory activity as compared to standard drug dexamethasone. The antioxidant activity of 1,3- Dimethyl 2,6-Diphenyl 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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime had powerful anti-inflammatory and *in vitro* antioxidant activity.

Keywords: Anti-inflammation, *In vitro* Antioxidant, 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime.

INTRODUCTION

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 [1]. 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 [2]. 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 to investigate the anti-inflammatory and antioxidant activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime.

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MATERIALS AND METHODS

Preparation of 1,3- Dimethyl 2,6-Diphenyl Piperidine 4-One:

A mixture of aqueous solution of methyl amine (40 %) solution (1 mole), benzaldehyde (1.5 mole) and the corresponding ketone (1 mole) in glacial acetic acid (20 ml) was heated to boiling and allowed to stand overnight . Addition of con.HCl yielded the hydrochloride. The free base obtained by neutralization of the hydrochloride suspended in acetone with aqueous ammonia followed by distillation was recrystallised from ethanol. Observed melting point was 128 °c.

PREPARATION OF OXIME

Preparation of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime :

The oxime was prepared from the respective ketones by the following procedure 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 hours. The reaction mixture was concentrated and the poured into water (300 ml). The solid obtained was filtered, washed with water and recrystallised from ethanol. Observed melting point was 179°c.

Experimental animal

Male albino rats of Wistar strain approximately weighing 180-190g 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 ± 2º C and 12 hour 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 [3]. After 12hrs 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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime at a dose of 100, 250 and 500 mg/kg orally. Group V was orally administered 2mg/kg (ip) Dexamethasone as a standard drug. The animals were pretreated with the 1,3- Dimethyl 2,6-Diphenyl 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 2½hours 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 [4,5].

$$\% \text{ of inhibition rate} = \frac{V_c - V_t}{V_c} \times 100$$

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 1,3- Dimethyl 2,6-Diphenyl 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 the standard.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada [6]. Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/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 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

Radical scavenging activity (%) = 100–(Ac-As/Ac)x 100

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 [7]. 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 spectro

photometer. The superoxide anion scavenging activity was calculated according to the following equation:

% Inhibition = $((A_0-A_1) / A_0 \times 100)$

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

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 [8].

Carrageenan induced inflammation is a biphasic phenomenon and is a useful model to detect oral actions of anti-inflammatory agents [9]. 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 [10].

The effect of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime on carrageenan induced paw oedema was calculated, and the result was presented in Table-1. The rat's foot pads become oedemateous after injection of carrageenan. Administration of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime reduces the paw oedema to inflammatory rats at a dose of 100, 200 and 500mg (kg body weight). The dose dependent a significant decrease of paw oedema and the reference drug dexamethasone (2mg/kg body weight) exhibited significant decrease. Among the various doses, the 500mg/kg (body weight) of 1,3- Dimethyl 2,6-Diphenyl 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 [11]. This study showed that all the doses of 1,3- Dimethyl 2,6-Diphenyl 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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime also effectively suppressed the inflammation produced by serotonin induced by hind paw edema, which indicates that the 1,3-Dimethyl 2,6-Diphenyl 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 1,3-Dimethyl 2,6-Diphenyl piperidine 4-One Oxime on carrageenan mediators-induced paw oedema may be related to inhibition of inflammation mediator formation.

***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 [12]. 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 expended to find effective antioxidants for the treatment or prevention of free radical-mediated deleterious effects [12].

DPPH Assay

Recently, the use of the $DPPH^{\bullet}$ 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 $DPPH^{\bullet}$ 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 $DPPH^{\bullet}$ is thought to be due to their hydrogen donating ability [12]. DPPH radical scavenging activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime and standard as ascorbic acid are presented in Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of

antioxidants [13]. The half inhibition concentration (IC_{50}) of ascorbic acid and 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime were 35.3µg ml^{-1} and 54.13 µg ml^{-1} respectively (Table 2 and fig 1)**.** The 1,3- Dimethyl 2,6-Diphenyl 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.

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, is very harmful to the cellular components in a biological system [13]. The superoxide anion radical scavenging activities of the 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime assayed by the PMS-NADH system were shown in Fig 2. The superoxide scavenging activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime was 44.03 µg ml^{-1} and ascorbic acid were 31.62µg ml^{-1} respectively (Table 2 and fig 2)**.** These results suggested that 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime had notably superior superoxide radical scavenging effects.

Table 1. Effect of 1,3- Dimethyl 2,6-Diphenyl 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)	28.77 ± 2.01	56.64 ± 3.96	63.19 ± 4.42	70.78 ± 4.95	72.15 ± 5.05
Group III (%) (200 mg/ml)	45.70 ± 3.19	65.18 ± 4.56	69.45 ± 4.86	72.52 ± 5.07	74.87 ± 5.24
Group IV (%) (500 mg/ ml)	43.06 ± 3.01	66.71 ± 4.66	71.75 ± 5.02	72.70 ± 5.08	75.95 ± 5.31
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.

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	15.42± 1.07	25.6±2.04	21.45±1.56	31.25 ± 2.50
40	28.65±.2.02	61.26±4.90	47.85±3.34	64.23 ± 5.13
60	61.54±4.30	88.98±7.11	71.57±5.20	89.54 ± 7.16
80	76.54±5.33	99.34±7.94	84.72±5.96	98.51 ± 7.88
IC_{50} (ml)	54.13	35.03	44.03	31.62

Fig 1. DPPH radical scavenging activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime

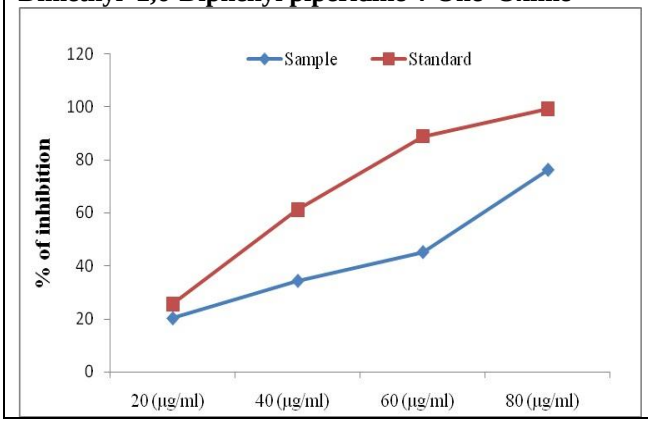
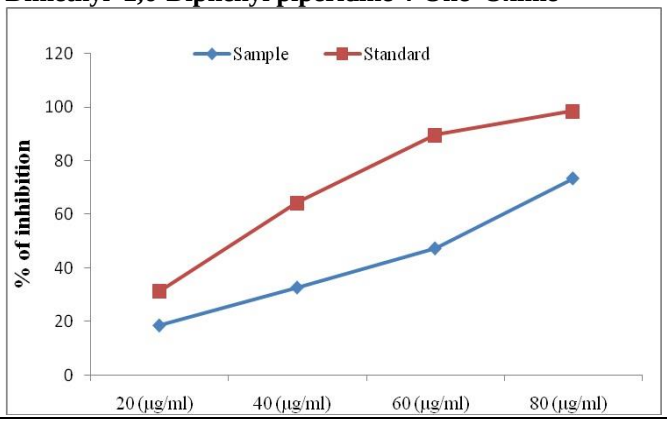


Fig 2. Superoxide radical scavenging activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime



CONCLUSION

In vitro anti-inflammatory and antioxidant activity of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime were tested. Results of the present study indicates that higher dose of 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime has potent anti-inflammatory activity close to standard drug. The above results confirmed that 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime possess significant anti-inflammatory activity as compared to standard drug dexamethasone. The antioxidant activity of 1,3- Dimethyl 2,6-Diphenyl 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 1,3- Dimethyl 2,6-Diphenyl piperidine 4-One Oxime had powerful *in vitro* anti-inflammatory and antioxidant activity.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Calixo JB, Campos MM, Otuki MF, Santos AS. Anti-inflammatory compounds from plant origion Part 2. Modulation of Pro-inflammatory cytokines, chemokianes and adhesion molecules. *Plant medica*, 70, 2004, 93-103.
2. Ghosh MN. Fundamentals of experimental pharmacology. Hilton & Co., Kolkata. 2008. Higgs G, Willam T. Inflammatory mediators, CRC press, New York, 1985.
3. Korycka DM, Richardson M. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and aminoacids. *Journal of Dairy Science*, 61, 1978, 400-407.
4. Krinsky NI. Mechanism of Action Biological Antioxidants. *Proc. Soc. Exp. Biol. Med*, 200, 1992, 248.
5. Liu F, Ooi CVE, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci*, 60, 1997, 763-771.
6. Meier R, Schuler W, Desaulles P. Leusic acid tumor inhibitor isolated from lichens. *Experimentation*, 6, 1950, 469-471.
7. Nuutila AM, Pimia RP, Aarni M, Caldenty KMO. Comparision of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chemistry*, 81, 2003, 485–493.
8. Reiter RJ. Robinson GD. Where Do Free Radicals Come From?Melatonin. *Bantam Book*, 1995, 24.
9. Shan Biren N, Nayak BS, Seth AK. Search of medicinal plants as a source of anti-inflammatory and anti-arthritic agents. *Pharmaconogy magazine*, 2, 200677-80.
10. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of *xanthum* on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40, 1992, 945–948.
11. Sindhu M, Abraham TE. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *Food and Chemical Toxicology*, 44, 2006, 198–206.
12. Velavan S. Free radicals in health and diseases- A Mini Review. *Pharmacologyonline Newsletter*, 1, 2011, 1062-1077.
13. Winter CA, Risley EA, Nuss GW. Carrageenan-induced oedema in hind paw of the rats as assay for anti-inflammatory drugs. *Proc Soc Expe Biol Med*, 111, 1962, 544- 547.