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Antioxidant activity of newly synthesised imidazole (2-(2-benzylidenehydrazinyl)-5, 5-diphenyl-1, 5-dihydro-4H-imidazol 4-one)

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ABSTRACT

The newly synthesized imidazole was evaluated for anti-oxidant activity. Chloro derivative of imidazole was synthesized. In three steps and has significant antidiabetic activity. Antioxidant activity is evaluated by estimating the free radical scavenging activity by DPPH method and nitric oxide method by. Invitro. whereas invivo antioxidant activity is evaluated by estimiating Enzyme catalase which catalyses hydrogen peroxide , lipid peroxidation by TBARS and reduced glutathione levels. Free scavenging activity of the cholro derivative increased with increasing dose. Catalase acitivity and glutathione reduction increased whereas lipid peroxidation decreased. The synthesized Chloro derivative has significant antioxidant activity.

Key words: free radicals; Reactive oxygen species; Antioxidants

INTRODUCTION

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2)[1]. The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction.[2] These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins [3]. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms [4] , [5]. While damage to proteins causes enzyme inhibition, denaturation and protein degradation [6] The free radicals are formed as by products of various endogeneous processes that can be stimulated by external factors such as irradiation and xenobiotics[7]. As a result of reduction, Reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and hydroxyl radical, are thought to be generated in aerobic organisms [8,9]. They mediate biochemical reactions and physiological effects and involve in cellular metabolism[10] . Anxtioxidants play important role in the treatment of degenerative or chronic diseases like atherosclerosis ,brain dysfunction ,immune system decline and cancer[11,12,13]. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins[14]. Antioxidants are the essential defense mechanism to protect the body against free radical damage. 2-(2-benzylidenehydrazinyl)-5,5-diphenyl-1,5-dihydro-4H-imidazol 4-one) (C22 H17 N4OCl) was synthesized in three steps[15], and the compound was found to have significant antidiabetic activity[16]

MAINTENANCE OF ANIMALS: (experiments were conducted at institution with 1662/po/a/12/ CPCSEA)

Albino Wistar rats and Albino Wistar mice were purchased from mahaveer enterprises. Hyderabad. The animals were acclimatized to the conditions by maintaining them at the experimental conditions for

about 7 days prior to dosing. Cage number and individuals marking on the tail to identify the animals. The animals were housed six per cage of same sex in polypropylene cages with bedding of paddy. Pellet chew feed standard diet under good management conditions and water *ad libitum* was provided to the animals at the temperature 20-25^{o}c and 12 hour each at dark and light cycle was maintained.

MATERIALS AND METHODS

DPPH Method: The reaction mixture consisted of adding 0.5ml of sample, 3ml of absolute alcohol and 0.3ml of DPPH solution(0.5mM) in ethanol. The color changes from deep violet to pale yellow .The absorbance was read at 517nm after 100 min of reaction .The percentage of inhibition is calculated using the formula
% inhibition = OD control -OD sample/OD control *100[17,18]

NITRIC OXIDE:
Nitric oxide radical scavenging: Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicate.[19]

Invivo antioxidant activity: (post mitochondrial supernatant)
The Liver was dissected out , washed immediately in ice cold cold saline, a homogenate(10%w/v) was prepared in chilled phosphate buffer(0.1M,PH 7.4) containing 0.15M KCl. The homogenate was centrifuged at 800 g for 5min at 4°c to separate the nuclear debris. The supernatant then obtained was centrifuged at 10,500 g for 20 min at 4°c to get the pms . it was used to estimate lipid peroxidation(TBARS),reduced glutathione(GSH) concentrations activities.
,catalase (CAT) activities.[20]

LIPID PEROXIDATION:
0.5 ml of PMS (post mitochondrial supernatant)
0.5 ml of tris Hcl buffer
1 ml of ice cold trichloro acetic acid
1 ml of TBA (Thiobarbituric acid)
1 ml of distilled water
absorbance at 532nm (spectrophotometer)
lipid peroxidation was assayed in the form of TBARS by the method of [21] (To 0.2ml of 8.1%(w/v) sodium lauryl sulphate , added 1.5ml of 20% v/v acetic acid soln. pH was adjusted to 3.5 with 5 N sodium hydroxide and 1.5ml TBA (0.8%w/v) to 0.2ml pms. The volume was made upto 4ml with distilled water .it was heated at 95 °c for 60min. after cooling 1ml distilled water and 5ml (n butanol &pyridine(15:1) was added and centrifuged at 4000rpm for 10min. Organic layer was taken and absorbance was measured at 532nm. extinction coefficient of 1.56x105M-1/cm -1 N was used to quantify TBARS.and expressed as moles of tbars per mg protein

CATALASE: Assay mixture is made with 1.95ml of phosphate buffer (having 0.05 molar concentration and PH-7.0) add 1ml of H_2O_2 (having 0.019 molar conc) ,to this add 0.05ml 0f post mitochondrial supernatant. The final volume will be 3ml(1.95+1+0.05) . Absorbance was measured at 240nm using spectrophotometer [22]

REDUCED GLUTATHIONE:
1 ml of PMS (post mitochondrial supernatant)
1 ml of Sulphosalicylic acid
cold centrifuge at 1200 rpm for 15 minutes
2.7 ml of phosphate buffer(1,2- dithio –bis-nitrobenzoic acid)(DTNB)
412 nm absorbance (spectrophotometer)

To 1ml of PMS (10%w/v) was precipitated with 1ml of sulphosalicylic acid.the samples were kept at 4°c for atleast 1 hr. centrifuged at 1200 g for 15 min at 4 was and centrifuged at cold at 1200rpm for 15 min. at 4 °c . the assay mixture containing 0.1ml filtered portion and 2.7ml phosphate buffer(0.1M) ,ph 7.4) and 0.2ml DTNB (100mM)in a total volume of 3ml. the yellow color developed . t was read at 412 nm on a spectrophotometer [20]

RESULTS AND DISCUSSION

DPPH scavenging activity is measured as percentage inhibition. The percentage inhibition increased with the increase in concentration. Nitric oxide is estimated by the procedure described by Sreejayan .,1996 free radical scavenging activity increased with the dose. Scavenging activity increased from 42.66+_1.453 for 25µg/ml to 80.75+_1.295.

Sodium nitroprusside serves as a source of free radicals. Antioxidants compete with oxygen leading to reduced formation of nitric oxide. The chromophore formation during the process of diazotization of the nitrite and coupling with naphthyl ethylene diamine us used as marker for

NO scavenging activity.[23] The absorbance decreases as the concentration of chloro derivative increases. Chloro derivative reduced the formation of chromophore by scavenging the NO in dose dependent manner.[24] Lipid peroxidation has been implicated in disease states such as atherosclerosis, irritable bowel disease, retinopathy of prematurity , bronchopulmonary dysplasia, asthma, Parkinson's disease, kidney damage, preeclampsia and others.[25].chloro derivative exhibited protection against lipid peroxidation. The enzyme catalase is an endogenous antioxidant present in all aerobic cells helping to facilitate the removal of hydrogen peroxide. The enzyme consists of 4 subunits of the same size, each unit consists of a heme active site to accelerate the decomposition of H_2O_2 to water and oxygen. (Catalase enzyme activity- uv assay). The test compound exhibited catalase activity more than control and standard. Test compound value is 42.49+_1.72, standard is 41.20+_1.183 where as control is 31.8+_1.220. Lipid peroxidation activity of chloro derivative TBARS levels are decreased . 38.74+_0.47($p<0.001$) in control to 28.57+_0.29($p<0.05$). Glutathione has several functions as endogenous antioxidant (Scholz RW1989) ,regulation of nitric oxide cycle [26,27], used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport,(drug.com) and enzyme activation and has vital function in iron metabolism[28,29]

CONCLUSION

The chloro derivative has significant antioxidant activity .with further studies the compound can be developed into lead molecule.

Table.1. Effect of Chloro derivative of Imidazole on DPPH in alloxan induced diabetic rats.

S. No:	Concentrations (µg/ml)	Dpph standard extract (% inhibition)	Dpph chloro derivative (% inhibition)
1	25	92.14±2.38	57.41±1.094
2	50	96.30±1.99	65.64±2.92
3	75	96.09±2.022	79.01±1.85
4	100		90.09±1.90
5	125	97.60±2.36	98.97±2.20

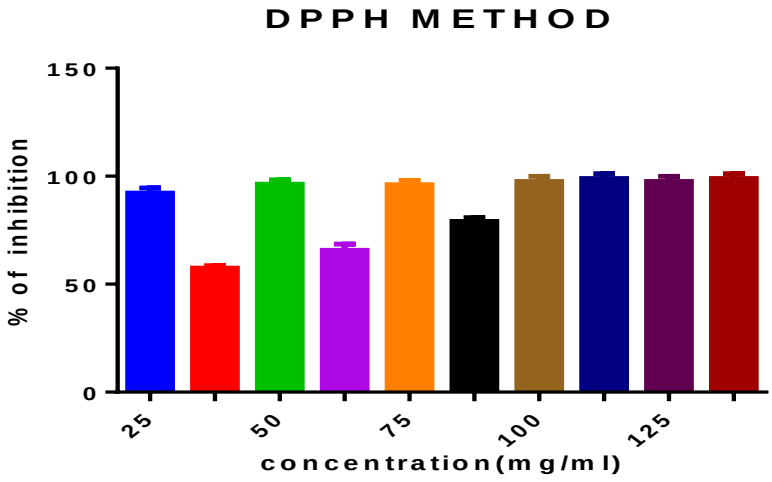

Figure 1:

Table 2: Effect of Chloro derivative of Imidazole on nitric oxide activity in alloxan induced diabetic rats.

S. No:	Concentrations µg/ml	Nitricoxide standard Ascorbic acid (%activity) inhibition)	Nitricoxide (Test) (%activity) inhibition)
1	25	72.08±2.639	42.66±1.453
2	50	76.38±2.529	56.43±2.338
3	75	81.173±2.228	63.546±2.314
4	100	87.203±1.584	72.113±2.206
5	125	91.706±1.674	80.75±1.295

Table 3. Effect of chloro derivative on Catalase activity in alloxan induced diabetes :

S. No:	Treatment	Catalase
1	Normal	52.70±1.354
2	Negative control	31.8±1.220
3	Standard	41.210±1.183
4	Test compound	42.49±1.72

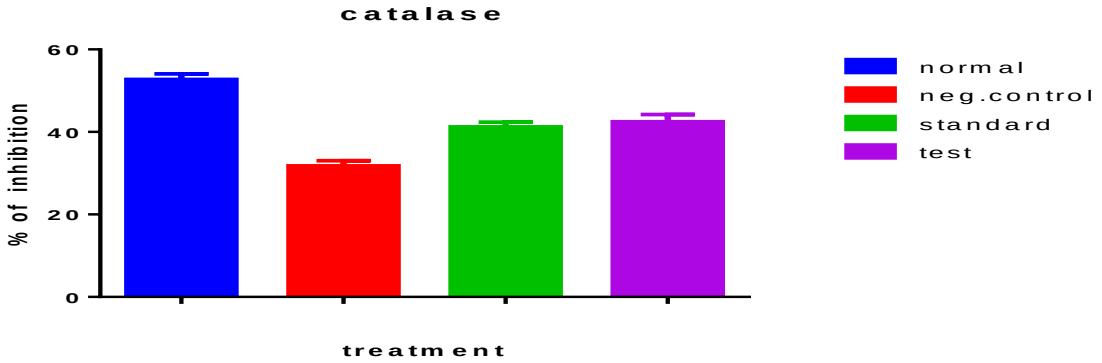

Figure 3.

TABLE 4.Effect of chloro derivatives of imidazole on antioxidant parameters LPO and GSH

Groups	Parameter	
	LPO (nMol /g wt)	GSH (m mole/g of tissue)
normal	21.67 ± 0.61	34.76 ± 0.29
Negative control	38.74 ± 0.47[a]	13.39 ± 0.70[a]
test (50mg)	28.57 ± 0.29[b]	24.19 ± 0.32[c]
standard (10mg)	25.42 ± 0.35[a]	28.57 ± 0.37[a]

One way ANOVA followed by Dunnett's test when compared with group B(NEGATIVE CONTROL)
[a]$P < 0.001$. [b]$P < 0.05$,[c]$P < 0.01$,

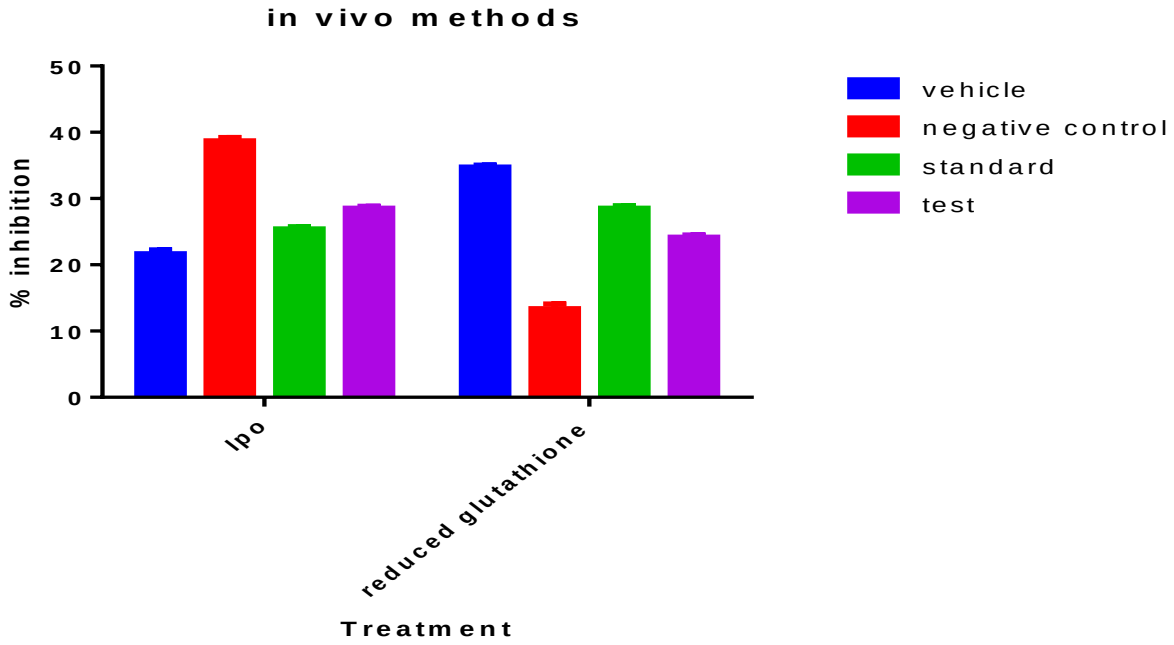

FIGURE 4

REFERENCES

1. Valko, M; Leibfritz, D; Moncol, J; Cronin, M; Mazur, M; Telser, J (2007). The International Journal of Biochemistry & Cell Biology **39** (1): 44 -84. .
2. Stohs, S; Bagchi, D (1995)., Free Radical Biology and Medicine **18**(2): 321–36.
3. Sies, Helmut (1997). , Experimental physiology **82** (2): 291–5.
4. Nakabeppu, Yusaku; Sakumi, Kunihiko; Sakamoto, Katsumi; Tsuchimoto, Daisuke; Tsuzuki, Teruhisa; Nakatsu, Yoshimichi (2006). Biological Chemistry **387** (4): 373–9. .
5. Valko, Marian; Izakovic, Mario; Mazur, Milan; Rhodes, Christopher J.; Telser, Joshua (2004). Molecular and Cellular Biochemistry **266** (1–2): 37–56.
6. wikipedia
7. Halliwell B, Gutteridge JMC (1989) Free Radicals in Biology and Medicine, Ed 2. Clarendon Press, Oxford
8. Mccord, J.M. N. Engl. J.Med. 312; 1985: 159-163.
9. Clark RA, Leidal KG, Pearson DW, Nauseef WM, J. Biol. Chem, 262, 1987, 4065-4074
10. Poli, G.; Leonarduzzi, G.; Biasi, F.; Chiarpotto, E. Curr. Med. Chem. 2004, 11, 1163-1182
11. Halliwell B (1991) : Am J Medicine 91: 14-22
12. Rice-Evans C., N.J. Miller, 1997, Free Radic. Res., 195, pp. 26-27
13. Behl C, Davis JB, Lesley R, et al. Cell. 1994;77:817–2
14. Aurelia Magdalena Pisoschi and Gheorghe Petre Negulescu., Biochem & Anal Biochem 1:106 October 31, 2011
15. Bethi Srinivas, G.E.Suhasini, Raju Bathula, Rama Devi, M.Nirmala*, Ramya Sree : : JAPS/Vol.2/Issue 1/2012
16. G.E. Suhasini , M. Nirmala, DR. Archanagiri, V. Varalaksmi and B. Solomon sunder Raj: IJPCBS 2013, 3(4), 1293-1304
17. Bondet V., Brand-Williams W., Berset C. Food Science and Technology. 1997; 30: 609-615
18. Brand Williams .W., Cuvelier M.E, and Berset C ., 1995 . Lebensmittel - Wissen s chaft und-Technology /Food Science and Technology 28 :23-30
19. N. Sreejayan, M.N.A. Rao, 1996, Drug Res, 46, pp. 169.
20. Rasool SN , Jaheerunnisa S, Jayaveera KN , Suresh Kumar ;International Journal of Applied Research in Natural Products Vol. 4 (1), pp. 1-10, Mar-April 2011

21. Ohkawa H, Ohishi N, Yagi K., Anal Biochem. , 1979 Jun;95(2):351-8.
22. Claiborne A. 1985. In: CRC Hand-book of methods for oxygen radical research, BocaRaton, ed. by R. A Greenwald. CRC Press: 283-284
23. Velavan S, Nagulendran K. Pharmacognosy Magazine 2007,3(9),26-33
24. Ilaversan R ,Mallika M etal, Afr J Trad CAM, 2005,(2),70-85
25. Mylonas C, Kouretas D.,.. In Vivo. 1999 May-Jun;13(3):295-309.
26. Clementi, Emilio; Smith, Guy Charles; Howden, Martin; Dietrich, Salvador; Bugg, S; O'Connell, MJ; Goldsbrough, PB; Cobbett, CS (1999. The Plant cell **11** (6): 1153–64
27. wikipedia
28. Chitranshu Kumar et al. Glutathione revisited: The EMBO Journal (2011) 30, 2044–2056.
29. wikipedia

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