International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 24-29
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF NOVEL THEOPHYLLINE DERIVATIVES

Devarakonda Srinivas[1], Devarakonda Venkatesh[1], Devarakonda Krishna Prasad[1*], Vasudha Bakshi[1] and Gangarapu Kiran[1]

[1]Department of Pharmacy, Anurag Group of Institutions, Venkatapur(V), Ghatkesar(M), Medchal(D), Telangana, India-500089.

***Corresponding Author Email: kittukp25@gmail.com**

ABSTRACT

The present investigation is to assess the free radical quenching potential of Novel Theophylline containing derivatives, using DPPH and Nitric oxide methods. The synthetic compounds C-16 and C-17 were used to assess the antioxidant activity were measured spectrophotometrically. The C-17 compound (78.7±0.608 in DPPH and 69.5±0.495 in Nitric oxide assay at 160ug/ml) showed more antioxidant potential than C-16 compound (73.4±0.515 in DPPH and 65.5±0.496 in Nitric oxide assay at 160 ug/ml). Both the C-16 and C-17 compounds have showed less Nitric oxide scavenging than the DPPH scavenging activity and were comparable with standard Ascorbic acid. The results obtained from the present study were showed that the C-16 and C-17 compounds exhibited potential source of antioxidant activity. The novel Theophylline derivatives were showed dose dependent inhibition of free radicals which might be due to the presence of purine ring system in their chemical structure, especially alkyl side chain in the C-17 compound.

KEY WORDS

DPPH, Nitric oxide, Radical scavenging activity and Theophylline

INTRODUCTION

Internally, free radicals are produced for example, hypochlorous acid scavenging (HOCl); hydroxyl radical scavenging (HO^- radical), hydrogen peroxide scavenging (H_2O_2), peroxyl radical scavenging (ROO^- radical) [1] as a normal part of metabolism within the mitochondria, through xanthine oxidase, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia, and physical exercise. External factors that help to promote the production of free radicals are smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone. It is ironic that these elements, essential to life (especially oxygen) have deleterious effects on the human body through these reactive species The balance between the production and neutralization of ROS by antioxidants is very delicate, and if this balance tends to the overproduction of ROS, the cells start to suffer the consequences of oxidative stress.[2] Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules that can cause damage to living cells and tissues in a process called "oxidative stress". that plays a decisive role in the development of various diseases. [1] The body uses vitamins and minerals to counteract the oxidative stress and which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged such compounds are called antioxidants. [3]

Theophylline is a methylxanthine, similar in structure to the common dietary xanthines caffeine and theobromine. Theophylline also known as 1,3-dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma under a variety of brand names. Although theophylline has been in clinical use for more than 70 years, its mechanism of action at a molecular level and its site of

action remain uncertain, although there have been important recent advances. Several molecular mechanisms of action have been proposed, many of which appear to occur only at higher concentrations of theophylline than are effective clinically. Xanthine derivatives, including theophylline, are known for important biological effects such as hypoglycemiant, bronchodilatory, anti-inflammatory, antioxidant and anticancer. [4] In this study, theophylline derivatives were evaluated for their *In-vitro* antioxidant effects.

MATERIALS AND METHODS

Materials

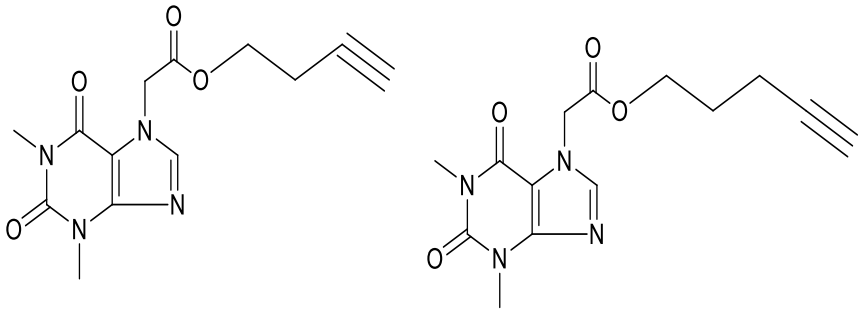
Drugs and chemicals

DPPH (from HIMEDIA Laboratories, Mumbai) Alcohol (Ethanol from FINAR chemicals, Hyderabad), Sodium Nitroprusside (from SD fine chem. Limited, Mumbai) Griess reagent (from sigma life sciences, Hyderabad) and Ascorbic acid (SD fine chem. limited, Mumbai)

Theophylline Derivatives: Theophylline containing acetylenes samples (C-16 and C-17) were gifted by Dr. G. Kiran Kumar (Department of Pharmaceutical Chemistry, Anurag Group of Institutions), Hyderabad and reported earlier in literature. All the synthesized analogs were confirmed by their melting point and were compared with the reported literature. [5]

C-16: but-3-yn-1-yl (1, 3-dimethyl-2, 6-dioxo-1, 2, 3, 6 tetrahydro-7-purin-7-yl) acetate.

C-17: but-3-yn-1-yl (1-dimethyl-2, 6-dioxo-1, 2, 3, 6 tetrahydro-7-purin-7-yl) acetate.



C-16 C-17

Fig 1: Structure of theophylline derivatives C-16 and C-17

Apparatus

U.V spectrophotometer (UV-3200, Labindia, 270V), Micropipettes (1000ul and 100ul) and weighing balance (Vignan instruments, Hyderabad).

Methods [6], [7]

DPPH (α, α--Diphenyl- β-picryl-hydrazyl) Method

The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [absorbance] at 517 nm after 30 min of reaction using a UV spectrophotometer. The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3mL). The scavenging activity percentage (AA %) was determined. [5] Different concentrations of compounds and reference standard 10, 20, 40, 80 and 160 ug/ml Ascorbic Acid was used as reference standard.

The percentage of inhibition can be calculated using the formula:

$$\text{Inhibition (\%)} = (A_o - A_1 / A_o) \times 100$$

Where, A_0 is the absorbance of control; A_1 is the absorbance of test.

Nitric Oxide Method

The reaction mixture consists of 1ml of test sample and 3ml of 5mM solution of sodium nitroprusside and 1ml of phosphate buffer pH 7.4. Now the reaction mixture is incubated for 120 min at 25^0C. Then, 1ml of the above solution is mixed with 1ml of griess reagent and incubated for 30min at 25^0C. Find out the absorbance of the above incubated solutions of various concentrations

at 572nm. Calculate the % radical scavenging activity by the following formula.

The percentage of inhibition can be calculated using the formula:

$$\text{Inhibition (\%)} = (A_o - A_1 / A_o) \times 100$$

Where, A_0 is the absorbance of control; A_1 is the absorbance of test.

STASTICAL ANALYSIS

The percentage inhibition of DPPH and Nitric oxide radicals in respective methods were expressed as Mean ± Standard Deviation, which were calculated by using Graph pad prism (version 7).

RESULTS AND DISCUSSION

DPPH Method

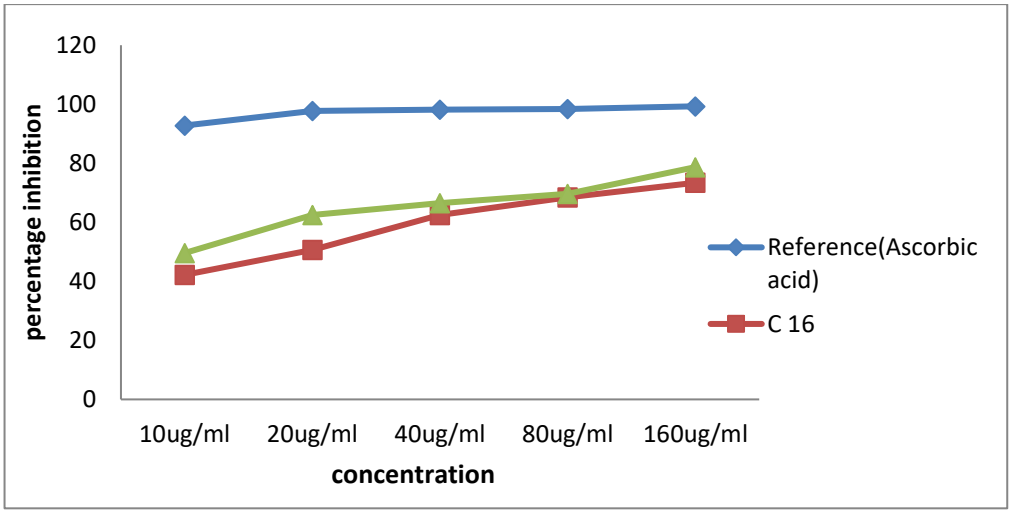
The result were given in the table 1 and represented in figure 2. The C-16 and C-17 compound showed increased radical scavenging activity with increased concentration. The effective free radical scavenging activity of C-16 (68.5±0.499 and 73.4±0.515) and C-17 (69.6±0.508 and 78.7±0.608) were found at 80 and 160 ug/ml respectively. The radical activities assay showed that results clearly indicate that the free radical scavenging activity of C-16 and C-17 compounds were showed radical scavenging activity when compared with that of Ascorbic acid (98.4±0.510 and 99.3±0.306) at 80 and 160 ug/ml respectively.

Table 1: DPPH radical scavenging ability of Theophylline derivatives

S.No	Conc.ug/ml	C-16		C-17		Ascorbic Acid	
		Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
1	10	0.4221	42.2±1.340	0.3734	49.6±0.616	0.0519	92.8±0.153
2	20	0.3634	50.7±0.621	0.2775	62.5±0.495	0.0148	97.7±0.641
3	40	0.2768	62.5±0.503	0.2468	66.5±0.502	0.0133	98.2±0.527
4	80	0.2339	68.5±0.499	0.2245	69.6±0.508	0.0127	98.4±0.510
5	160	0.0123	73.4±0.515	0.1571	78.7±0.608	0.0059	99.3±0.306

Values are expressed as (Mean ± S.D), n=3. All the test compounds results were compared with the Standard (Ascorbic acid).

Fig 2: Comparative Analysis of Anti-Oxidant Activity of Theophylline derivatives (DPPH Method)



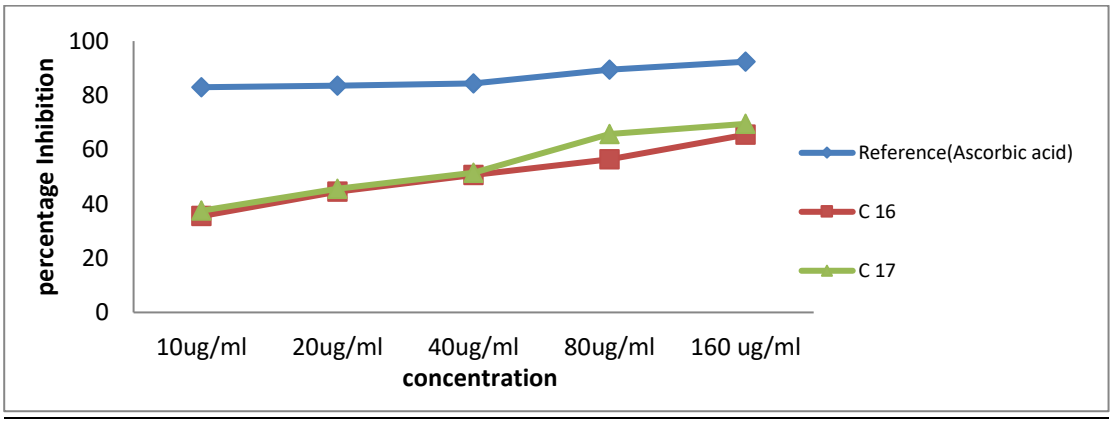
Nitric oxide assay

Table 2: Nitric Oxide radical scavenging ability of Theophylline derivatives.

S.No	Conc.ug/ml	C-16		C-17		Ascorbic Acid	
		Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
1	10	0.5080	35.5±0.500	0.6166	37.50±0.292	0.1570	83.0±0.500
2	20	0.4359	44.5±0.503	0.4605	45.6±0.539	0.1490	83.6±0.546
3	40	0.3878	50.6±0.518	0.4127	51.5±0.495	0.1475	84.4±0.556
4	80	0.3437	56.4±0.502	0.2900	65.7±0.586	0.0979	89.50±0.498
5	160	0.2714	65.5±0.496	0.2597	69.5±0.495	0.0700	92.42±0.497

Values are expressed as (Mean ± S.D), n=3. All the test compounds results were compared with the Standard (Ascorbic acid).

Fig 3: Comparative Analysis of Anti-Oxidant Activity of Theophylline derivatives



The result were given in the Table 2 and represented in figure 3. The two novel theophylline derivative compounds C-16 and C-17 compound showed increased radical scavenging activity with increased concentration i.e., they showed a dose-dependent increase in activity. The effective free radical scavenging activity of C-16 compound (56.4±0.502 and 65.5±0.496) and C-17 compound (65.7±0.586 and 69.5±0.495) were found at 80 and 160 ug/ml respectively. The radical activities assay showed that results clearly indicate the free radical scavenging activity of C-16 and C-17 compounds when compared with that of Ascorbic acid (89.50±0.498 and 92.50±0.497), at 80 and 160 µg/ml respectively.

DISCUSSION

In this study, we determined the free radical scavenging capacities of the two novel theophylline derivative compounds by using DPPH and Nitric Oxide *In-vitro* methods and widely used to determine the antioxidant potential, as they require relatively standard equipment and deliver fast and reproducible results. [8]

DPPH free radical scavenging activity

In this DPH assay, the reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. Table-1 shows the percentage of DPPH radical scavenged by two novel theophylline derivative compounds (C-16 and C-17) at various concentrations (Table 1 and Fig 2), the decreased in concentration of DPPH radical due to the scavenging ability of standard Ascorbic acid as reference compound. The synthetic compounds and the standard (Ascorbic acid) neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH, thereby changing the colour from purple to the yellow coloured stable diamagnetic molecule diphenyl picryl hydrazine. [9] The degree of discoloration of the DPPH solution indicated the scavenging potential of the novel theophylline derivatives in term of hydrogen donating ability. They showed substantial antioxidant activity in a dose-dependent manner similar to that of ascorbic acid which was used as a reference standard. [6] Maximum inhibition of the DPPH radical was observed when 160 µg/ml of the novel theophylline derivatives.

Further, the C-17 showed more antioxidant potential than the C-16 compound as per the values. (Table 1 and Figure 2).

Nitric oxide free radical scavenging activity:

In this Nitric oxide assay, the Nitric oxide ($NO^{\bullet}$) is an important chemical mediator generated by endothelial cells, macrophages and neurons**;** [10] it is involved in the regulation of various physiological processes. Excess concentration of NO^{-} is associated with several diseases. NO^{-} is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of NO^{-} via free electron oxidative reaction. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Incubation of solutions of sodium nitroprusside in PBS (Phosphate buffer solution) at 25°c for 2 h resulted in linear time dependent nitrite production, which can be reduced by the two novel theophylline derivatives.[11] Figure 3 shows the comparative NO^{-} scavenging activity of the two novel theophylline derivatives, this may be due to the antioxidant principles in the theophylline derivatives, which compete with oxygen to react with nitric oxide there by inhibiting the generation of NO^{-} radicals. Many theophylline and theobromine are known to become metabolized to their corresponding 8-oxo derivatives in the mammalian system and function as free radical scavengers suggesting their antioxidant effects. [12] From the above statement, it was known that the novel theophylline derivatives exhibit better antioxidant property was showed dose dependent inhibition of free radicals which might be due to the presence of purine ring system in their chemical structure, especially alkyl side chain in the C-17 compound, but the C-17 showed more antioxidant activity and this might be due to the substitution of the methyl group on the purine ring.

The imbalance between reactive oxygen species and antioxidant defense mechanism leads to oxidative modifications in cellular membranes or intracellular molecules. ROS are continuously produced during normal physiological events and are removed by antioxidant defense mechanisms [13]

CONCLUSION

The both novel theophylline derivative compounds (C-16 and C-17) were exhibited the antioxidant activity in progressive manner i.e. increase in radical scavenging with increase in concentration against DPPH and Nitric oxide radical. The C-17 compound showed more radical scavenging activity when comparative to that of C-16 compound with reference to Ascorbic acid as standard, the present study was showed that the C-16 and C-17 compounds exhibited potential source of antioxidant activity which might help us in preventing the progress of oxidative stress and other degenerative diseases like atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye disease.

ACKNOWLEDGMENT

The authors are very thankful to Anurag Group of Institutions, School of Pharmacy, for financial assistance of the work, providing infrastructure facilities and carried out antioxidant of novel theophylline derivatives.

REFERENCES

[1]. Praveen Singh, Ranjeet Kumar, Shachi Tiwari, Ranjana Khanna, Ashish Kumar Tewari and Hari Dev Khanna "Docking, Synthesis and Evaluation of Antioxidant Activity of 2,4,5-Triaryl Imidazole" *Clin Med Biochemistry*, 1(1): 1-4, (2015)

[2]. Lobo V., Phatak A and Chandra N. "Free radicals and functional foods: Impact on human health". *Pharmacogn. Rev*, 4: 118-126, (2010)

[3]. Poljsak B, Dusan S, and Irina M, "Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants" *Oxidative Medicine and Cellular Longevity*, 1-11, (2013)

[4]. Peter J. B, "Theophylline" *Pharmaceuticals*: 3: 725-47, (2010)

[5]. Mensor L, Menezes FS, Leitao GG, dos Santos T and coube S "Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method" *Phytother res* 15, 127-130: (2001)

[6]. Francis Awah M. and Andrew Verla W., "Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf Extract"*J. Med. Plant. Res*: 4(24): 2479-87, (2010)

[7]. Brand-Williams W., Cuvelier M. E and Berset C.. "Use of a Free Radical Method to Evaluate Antioxidant Activity" *Lebensm.-Wiss. u.-Technol,* 28(1): 25-30, (1995)

[8]. Sudha R., CharlesKanakam C. and Nithya G,"In Vitro Antioxidant Activity Of Different Substituted Benzilic Acid Using 2,2-Diphenyl-1-Picryl Hydrazyl Radical, ABTS Assay Method" *Asian J Pharm Clin Res*, 9(3): 127-30, (2016).

[9]. H.A. Moharram , M.M. Youssef, "Methods for Determining the Antioxidant Activity: A Review" *Alex. J. Fd. Sci. & Technol.* 2014; 11(1); 31-42.

[10]. Rozina P, Sukalayan Kumar K and Pijush Saha S ,"In Vitro Nitric Oxide Scavenging Activity Of Methanol Extracts Of Three Bangladeshi Medicinal Plants" *The Pharma Innovation Journal*,: 1(12): 83-88, (2013)

[11]. Vadiraja B and. Madyastha K. M. "Antioxidant and Radical Scavenging Properties of 8-Oxo Derivatives of Xanthine

Drugs Pentoxifylline and Lisofylline" *Biochemical and Biophysical Research, 8*(5): 1212–17, (2001)

[12]. R. Rahul, Rakesh Kumar Jat, J. Saravanan "Synthesis, and *in-vitro* antioxidant activity of novel 1, 3, 4- oxadiazole-2-thione" *JIPBS,* 3 (3): 114-22, (2016)

Received:02.05.18, Accepted: 05.06.18, Published:01.07.2018

[13]. Shyamsunder A., Ram Narsimha reddy A., Krishna Prasad D., Poorna chander K and Sridhar V. "Free radical scavenging activity of ethanolic extract of trianthema Portulacastrum Linn" (Aizoaceae)**.** *Int. j. pharm. Sci.,* 2010: 2(2); 589-592.

***Corresponding Author:**
Devarakonda Krishna Prasad*
Email: **kittukp25@gmail.com**