Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 547 - 552

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN VITRO EVALUATION OF ANTI-OXIDANT PROPERTY OF TAPENTADOL

DR S. NAVEEN*, DR P. ELANGO AND DR D. CHELLATHAI

Department of Pharmacology, Sri Ramachandra Medical College & Research Institute, Chennai -600116

ABSTRACT

Our main objective of this study was to evaluate the antioxidant properties of Tapentadol. Antioxidant properties were evaluated using *In Vitro* DPPH [2, 2-diphenyl-1-picryl hydrazyl] and Nitric oxide free radical scavenging assays. Tapentadol was taken in the following concentrations 50 mg/ml, 25 mg /ml, 12.5 mg /ml, 6.25 mg /ml, 3.125 mg /ml while Ascorbic acid was used as Standard. Tests were performed in triplicate and results are expressed as Mean Tapentadol exhibits good dose dependent antioxidant activity at concentrations 50 mg/ml, 25 mg /ml, 12.5 mg /ml, 6.25 mg /ml, 3.125 mg /ml. Antioxidant activity is better in Nitric oxide free radical scavenging assay when compared to DPPH free radical scavenging assay which may point out radical specific scavenging activity of Tapentadol. Both DPPH and NO free Radical scavenging assays demonstrate that Tapentadol has antioxidant properties. The antioxidant property may be explored along with its established analgesic activity in chronic diseases.

KEYWORDS: **Tapentadol, DPPH, Nitric oxide, Free radicals and antioxidant.**

*Corresponding author

DR S. NAVEEN
Department of Pharmacology, Sri Ramachandra Medical College & Research Institute, Chennai -600116

1. INTRODUCTION

Tapentadol is an Opioid analgesic. It is centrally acting and has two mechanism of actions, μ opioid receptor agonism and Nor-Epinephrine reuptake inhibitor (increasing norepinephrine levels in brain)[1]. Tapentadol is used as an analgesic in acute as well as chronic moderate to severe painful conditions like osteoarthritis, rheumatoid arthritis, neuropathy and many [2]. Chemically tapentadol is 3- [(2R, 3R)-1-(dimethyl amino)-2-methylpentan-3-yl] phenol. OR 3- [(1R, 2R)-3-(dimethyl amino)-1-ethyl-2-methylpropyl] phenol [3].

Structure of Tapentadol

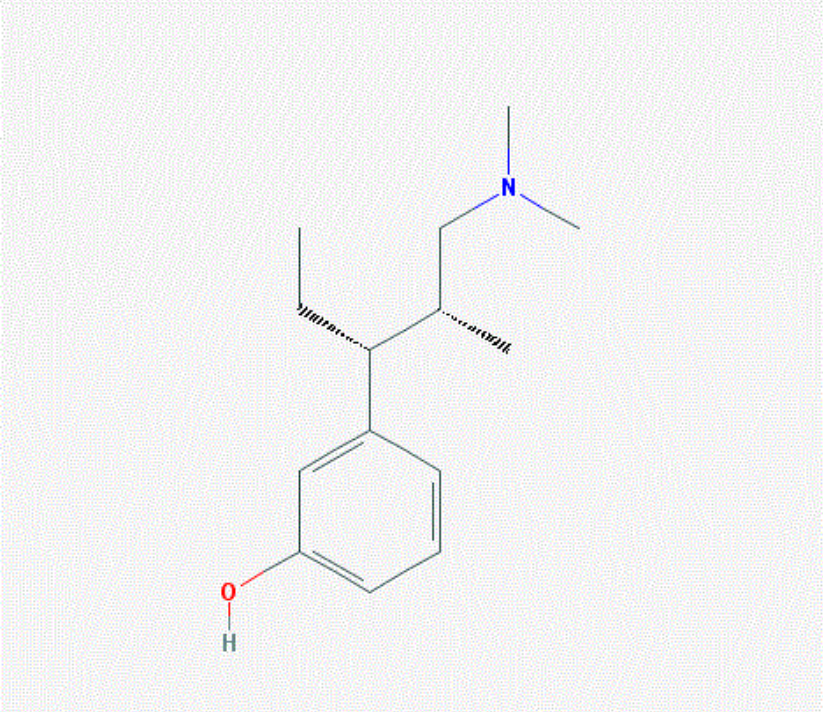


Figure no 1
Structure of tapentadol [3]

As seen in the chemical name and structure of tapentadol, it has a hydroxyl group (-OH)/phenolic group.Anti-oxidant activity or free radical scavenging is one of the very well-known properties of phenolic compounds [4]. In chronic painful conditions like osteoarthritis/ other degenerative joint diseases it is seen that there is increased production of reactive oxygen species (like super oxide anion) and there is a decrease in oxygen scavengers (like super oxide dismutase)[5]. Compounds that aid in scavenging these oxidative free radicals can be very useful in therapy of this chronic degenerative articular pain. Since, chronic conditions like osteoarthritis, rheumatoid arthritis etc. where tapentadol is used for its analgesic effect has oxidative stress as one of the basis of its pathology [5], this study aims to evaluate the invitro anti-oxidative properties of tapentadol using free radical scavenging assay.

2. MATERIALS

Tapentadol was procured from Chandran enterprises Chennai while DPPH from Sigma Aldrich. Double distilled water was obtained from the Milli-Q system. The study was conducted in Department of Pharmacology, Sri Ramachandra Medical College and Research Institute.

3. METHODS

3.*1 DPPH FREE RADICAL SCAVENGING ASSAY*

PRINCIPLE [6]

The DPPH assay method is based on the reduction of DPPH, a stable free radical. DPPH solution is purple in colour (maximum absorption at 517nm) as it has a free/an odd electron. When antioxidants react with DPPH, the stable free radical becomes paired off in the presence of a hydrogen donor and gets reduced to the DPPH-H and as a consequence the absorbance gets decreased from the DPPH radical to the DPPH-H form resulting in decolourization (yellow colour) which is proportional to the number of electrons paired.

PROCEDURE

DPPH radical scavenging activity was done using the method of Yohozowa et al**.** [7].The reaction mixture contains 1.9ml of DPPH solution (80µ/ml in methanol) with aliquots of 0.1ml of different concentrations of tapentadol in double distilled water (3.125 mg /ml, 6.25 mg /ml, 12.5 mg /ml, 25 mg /ml, 50 mg/ml) .The mixture was shaken and incubated in dark for 30 min at room temperature. Similarly five test tubes were incubated with DPPH and Ascorbic acid in the same concentration (50 mg/ml, 25 mg /ml,12.5 mg /ml, 6.25 mg /ml, 3.125 mg /ml), which was considered as the standard. Control for Tapentadol and ascorbic acid was taken as 1.9ml of DPPH solution (80µg /ml) with 0.1 ml of double distilled water. The resultant absorbance was recorded at 517nm using ELICO Double beam- SL210 UV-VIS Spectrophotometer

The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

All tests were carried out in triplicates and the mean readings were noted

3.2 NITRIC OXIDE FREE RADICAL SCAVENGING ASSAY

PRINCIPLE

The principle of nitric oxide radical scavenging assay is to evaluate the effectiveness of the test compound to neutralize the free nitric oxide radicals which is formed. In order to quantify this effect Griess reagent is added to the reaction mixture after incubation. Griess reagent has two components, one is the sulphonamide part and the other one is Azo dye agent which is naphthyl ethylenediamine dihydrochloride (NEDD).When sulphanilic acid is added (sulphonamide), there is a diazonium salt formation with the nitrites present in solution[8]. Next when the Azo dye agent NEDD is added, there is development of a pink colour tinge in the reaction mixture which is proportional to the quantity of diazonium salt present. In commercially available Griess reagent 2-naphthylamine is used which is classified as an acutely toxic potent carcinogen and moreover 2-Naphthylamine diamine forms a more polar and hence a much more soluble dye in acidic aqueous medium. Hence 2-Naphthylamine was replaced by 0.2% NEDD when prepared in-house.

PROCEDURE

The nitric oxide radical scavenging activity was done using the method of Alderton et. al[9]. A solution containing sodium nitroprusside in the conc. of 10mM was prepared in phosphate buffered solution. Tapentadol was dissolved in double distilled water and solutions of the following concentrations were prepared i.e. (3.125 mg /ml, 6.25 mg /ml, 12.5 mg /ml, 25 mg /ml, 50 mg/ml) respectively. Aliquots of 2ml of sodium nitroprusside (10mM in phosphate buffered saline) were mixed with 1ml of Tapentadol (3.125 mg /ml, 6.25 mg /ml, 12.5 mg /ml, 25 mg /ml, 50 mg/ml) and incubated in dark for 4 hours at 37^0C. Similar concentrations of ascorbic acid was used which was considered as the standard. Similarly control was also incubated by replacing Tapentadol with equal volume of its solvent i.e. double distilled water. After 4 hours of incubation, into the incubated solutions, 0.5ml of Griess reagent was added and the absorbance was read at 546nm using ELICO Double beam- SL210 UV-VIS Spectrophotometer.

The percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

All tests were carried out in triplicates and the mean readings were noted

4. RESULTS

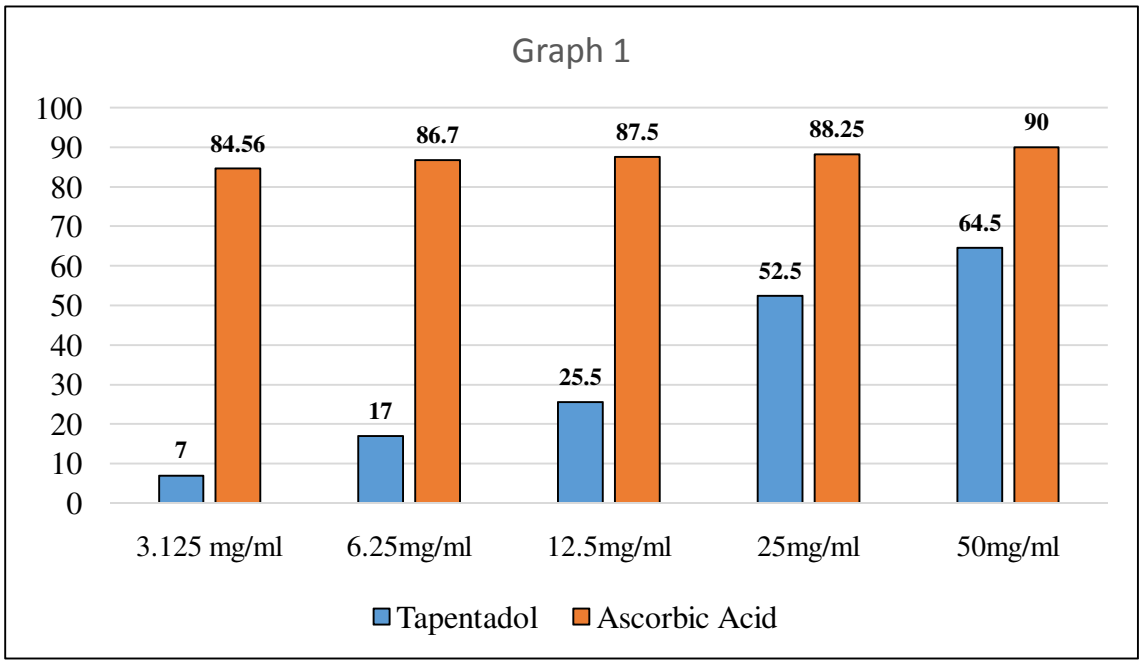
4.1 DPPH Free Radical Scavenging Assay

Tapentadol at concentrations of 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml exhibited 7%, 17%, 25.5 %, 52.5 % and 64.5 % DPPH free radical scavenging activity respectively while Ascorbic acid at concentrations of 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml exhibited 84.56%, 86.7%, 87.5 %, 88.5 % and 90 % DPPH free radical scavenging activity respectively.

Table no 1
Percentage Inhibition of DPPH Free Radical

Concentration (mg/ml)	Percentage inhibition by Tapentadol	Percentage inhibition by Ascorbic Acid
3.125 mg/ml	7 %	84.56 %
6.25 mg/ml	17 %	86.7%
12.5 mg/ml	25.5 %	87.5 %
25 mg/ml	52.5 %	88.25 %
50 mg/ml	64.5 %	90%

Graph 1
Percentage Inhibition of DPPH Free Radical



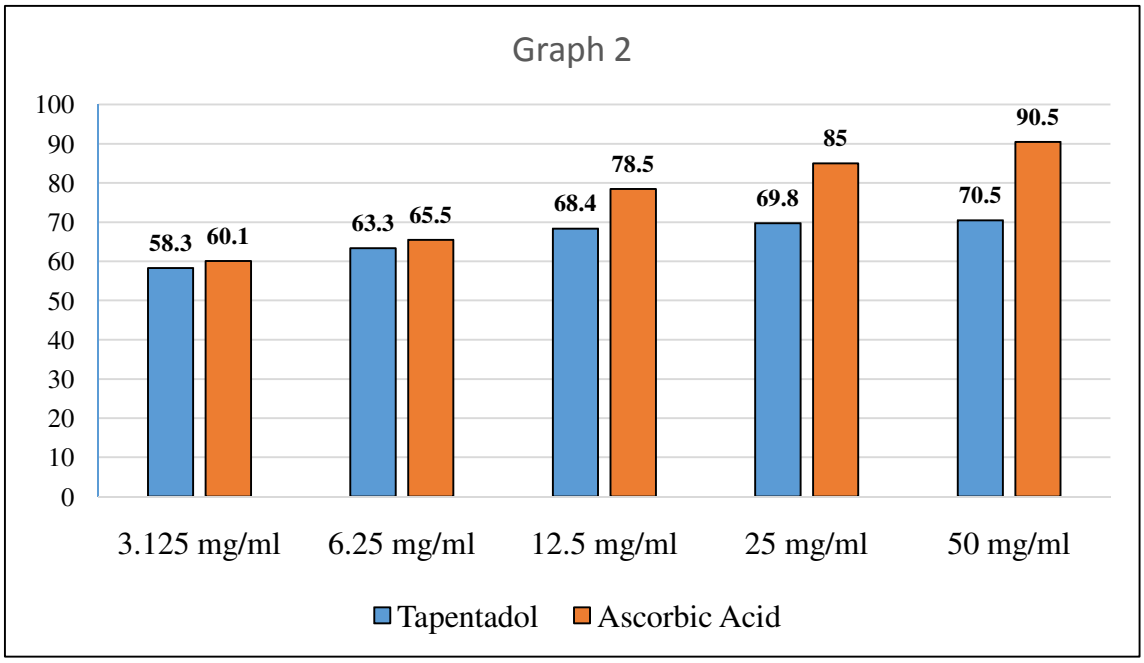
4.2 NITRIC OXIDE FREE RADICAL SCAVENGING ASSAY

Tapentadol at concentrations of 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml exhibited 58.3%, 63.3%, 68.4 %, 69.8 % and 70.5 % Nitric oxide free radical scavenging activity respectively while Ascorbic acid at concentrations of 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml exhibited 60.1%, 65.5%, 78.5 %, 85 % and 90.5 % Nitric oxide free radical scavenging activity.

Table no 2
Percentage Inhibition of Nitric Oxide Free Radical

Concentration (mg/ml)	Percentage inhibition by Tapentadol	Percentage inhibition by Ascorbic Acid
3.125 mg/ml	**58.3 %**	**60.1 %**
6.25 mg/ml	**63.3 %**	**65.5 %**
12.5 mg/ml	**68.4 %**	**78.5 %**
25 mg/ml	**69.8 %**	**85 %**
50 mg/ml	**70.5 %**	**90.5%**

Graph 2
Percentage Inhibition of Nitric oxide Free radical



5. DISCUSSION

Tapentadol is used in various acute and chronic painful conditions for its analgesic activity[10] (opioid). DPPH and Nitric oxide free radical scavenging assay are used to assess antioxidant properties of various compounds[11]. As there are limited literatures on the antioxidant properties of tapentadol, this study will help in evaluating additional antioxidant properties of tapentadol. As seen from the results tapentadol exhibited a dose dependent increase in the free radical scavenging activity. Activity was evident at lower doses of 3.125 mg/ml and showed a steady rise over to 50 mg/ml, which was the maximum tested concentration. Activity of tapentadol was compared with Ascorbic acid (i.e. the standard in this study) and was seen that the free radical scavenging activity of Tapentadol was comparable with that of Ascorbic acid at higher concentrations. It can be seen that ascorbic acid does not show a significant difference in its antioxidant activity at the range of concentrations selected for this study but in order to maintain a comparative note with that of Tapentadol, lower concentration of ascorbic acid was not included. It was also seen that Tapentadol exhibited better Nitric oxide free radical scavenging activity when compared with its DPPH free radical scavenging activity (refer table 1 and table 2), which may point out radical specific scavenging activity of Tapentadol.

6. CONCLUSION

Tapentadol has exhibited free radical scavenging property on both DPPH and Nitric oxide free radicals. This property can be one of the several basis by which Tapentadol exerts its pharmacological effects in diseases which have chronic inflammation as its

pathological basis like osteoarthritis [12], rheumatoid arthritis [13]. Further studies can be done and momentum must be gained in studying the antioxidant and anti-inflammatory properties of tapentadol which can aid in better utility of Tapentadol in the therapeutic armamentarium of chronic inflammatory disease.

7. ACKNOWLEDGEMENT

I gratefully acknowledge Department of Pharmacology, Sri Ramachandra Medical College and Research Institute for providing facilities and all the faculty of the department who helped me carry out this work.

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