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Original Research Paper

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ANTIOXIDANT AND FREE RADICAL SCAVENGING EFFECT OF PRUCALOPRIDE : AN INVITRO STUDY

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ABSTRACT

Introduction: Reactive oxygen species like superoxide radicals, hydroxyl radicals, singlet oxygen are implicated in causing tissue injury and cytotoxicity. They play a vital role in various diseases like ischaemic disease, degenerative disease and many more. Prucalopride is a novel drug used in chronic constipation that acts as selective, high affinity 5-HT4 receptor agonist which targets the impaired motility. This in-vitro study was done with an objective to evaluate additional radical scavenging property of the drug prucalopride.

Materials and Methods: In-vitro DPPH assay and Nitric oxide scavenging assay.

Results: The methanol extract of the drug Prucalopride showed free radical scavenging effect with DPPH at the concentration of 1000 µg/ml. And also an increase in nitric oxide scavenging effect was observed with increased concentration of drug.

KEYWORDS : DPPH, Nitric oxide, Constipation,

BACKGROUND:

Oxidative stress is a complex mechanism that arises due to the imbalance in the human antioxidant status. Reactive oxygen species (ROS) like superoxide radicals, hydroxyl radicals, singlet oxygen are formed as a result of several complex reactions that occur in an individual due to biological, chemical and various exogenous factors.[1] These radicals causes tissue injury and cytotoxicity. The same has been implicated in ageing and in a number of human diseases such as cancer, atherosclerosis, rheumatoid arthritis, hypertension, ischaemic disease, Alzheimer's disease, Parkinsonism and many others.[2] The protective antioxidant property offered by several fruits and vegetables and also by phenolic compounds and flavonoids has been studied.[3] This in-vitro study was undertaken to study the antioxidant effect of a drug prucalopride.

Chronic constipation is a common condition in our population especially in elderly age group and in Diabetic patients, which is difficult to treat. Diet counseling and increasing fibre content play a role in relief of symptoms for few patients. Poor adherence to proper meal plan, excess intake of fast food and fried food, stress, also contribute to this condition. Various laxatives and also newer pharmacological agents like cisapride are used in the therapy of chronic constipation. Prucalopride is one such novel drug used. It is a drug acting as a selective, high affinity 5-HT4 receptor agonist which targets the impaired motility associated with chronic constipation, thus nomalising bowel movements. Prucalopride alters colonic motility patterns via serotonin 5-HT4 receptor stimulation: it stimulates colonic mass movements, which provide the main propulsive force for defecation.[4] Prucalopride has also been tested for the treatment of chronic intestinal pseudo-obstruction. It is the only agent which is recommended by the National Institute for

Health Care Excellence (NICE) for chronic constipation in women.[5] Antioxidant property of the drug can play a role in Diabetic gastroparesis and inflammatory bowel disease.[6] Hence this in-vitro study was conducted to throw light on the antioxidant property of prucalopride.

OBJECTIVE:

To evaluate the antioxidant effects of the drug Prucalopride with DPPH, Nitric Oxide assay by in-vitro method.

MATERIALS AND METHODS:

1.Properties and procedure of the DPPH assay:

Action of DPPH:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Representing the DPPH radical by Z and the donor molecule by AH, the primary reaction is

$$Z\bullet + AH = ZH + A\bullet$$

where ZH is the reduced form and A is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.

Chemicals:

1. 1,1 – diphenyl -2- picrylhydrazyl (DPPH)
2. Dimethylsilphoxide (DMSO)
3. BHT (standard)-1.6mg/ml in methanol
4. Samples desired concentration from 1 mg /ml –max of 5mg / ml (in /DMSO)

Procedure of DPPH Assay:

3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. 100µl of BHT was added to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Then all test tubes were incubated at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

CALCULATION:

% Antioxidant activity

$$= \frac{\text{(Absorbance at blank) - (Absorbance at test)}}{\text{(Absorbance at blank)}} \times 100$$

2. SCAVENGING OF NITRIC OXIDE RADICALS

Principle:

Sodium nitroprusside in an aqueous solution form and at physiological pH spontaneously generates nitrite oxide which interacts with oxygen to produce nitrite ions, which can be measured at 550nm by spectrophotometer in the presence of Griess reagent.

Reagents and chemicals:

1.5mM Sodium Nitroprusside,

2.Griess reagent (1 part of 1% sulphonil amide and 1 part of 0.1% N 1naphthylethylenediamine in 2% orthophosphoric acid),

3. Phosphate buffer (pH- 7.4).

Procedure:

Sample was dissolved in distilled water for this quantification. Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025m, pH 7.4) was incubated with different concentration (100-400µg/ml) of methanol extract and tubes were incubated at 29°C for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents.

The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthyl ethylene diamine hydrochloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. Calculated the % inhibition by formula and plot graph in compared to standard.

Calculation:

$$\% \text{ inhibition} = \frac{\text{O.D.of control - O.D. of Test}}{\text{O.D. of control}} \times 100$$

Results

DPPH Scavenging Activity

The antioxidant activity was evaluated using DPPH assay and Nitric oxide assay. The methanol extract of the drug Prucalopride showed free radical scavenging effect with DPPH at the concentration of 1000 µg/ml. Ascorbic acid used as control also showed the antioxidant effect at this concentration. (Table 1)

Table 1: In-vitro Antioxidant activity of drug Prucalopride extract by DPPH Assay

	CONCENTRATION (µg/ml)	O.D	DPPH Activity (%)
Sample	1000	0.798	18
Ascorbic acid	1000	0.971	97.07

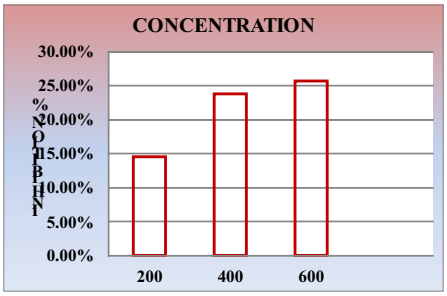
Nitric Oxide Scavenging Activity

Table 2 depicts the antioxidant effect of drug by Nitric oxide scavenging activity. The methanol extract of the drug prucalopride showed free radical scavenging effect with this assay. And also an increase in the activity was observed with increase in concentration of the drug.(Figure 1) The tested control ascorbic acid also showed anti-oxidant property.

Table 2:In-vitro Antioxidant activity of drug Prucalopride extract by Nitric Oxide Assay.

Drug Prucalopride			
Concentration(µg/ml)	200	400	600
O.D	0.259	0.231	0.225
Inhibition %	14.52%	23.76	25.74
Ascorbic acid : 96.26 for 100µg/ml Control O.D : 0.303			

Figure 1: Increase in the Antioxidant activity with increased concentration of drug Prucalopride extract



DISCUSSION:

The methanol extract of the drug Prucalopride was studied for free radical scavenging activity by DPPH assay. The inhibition % was found to be 18% with DPPH assay showing the antioxidant activity of drug at 1000µg/ml. The reduction capacity of DPPH radicals was found out by decreased absorbance at 517nm, which can be induced only with the antioxidants. Such property of decreased concentration of DPPH radical can be due to antioxidants was similar to the observation by Ganapaty S et al.[7]

Nitric oxide is an reactive nitrogen species synthesized by liver and considered as an important inter and intracellular signaling molecule which is essential for homeostasis.[8] In this study the Nitric oxide scavenging activity was found to be significant which also increased with increasing concentration of the drug.

To conclude, this invitro study provides useful information about the drug Prucalopride which is used for constipation. This study reveals the free radical scavenging activity of the drug prucalopride which can be an additional beneficial information regarding the drug. The superior action of this drug in constipation may also be attributed to the antioxidant activity of the drug. This drug can be further studied as an antioxidant molecule.

REFERENCES:

1. Aruoma,OI.(1994). Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxicol,32(7), 671-83. URL: https://www.ncbi.nlm.nih.gov/pubmed/8045480
2. Somasundaram. G, Inbaraj SD,Madhusudhanan N, Konda VGR, Madhavi E.(2012). Antioxidant and free radical scavenging activity of Nymphbea stellata : in vitro study. Journal of Pharmacy Research, 5(3),1350-52. URL: http://jprsolutions.info/newfiles/journal-file-56af547438f0b3.98075618.pdf
3. Chanda S, Dave R.(2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An review. Afr J Microbiol Res, 3(13),981-96.URL:https://www.researchgate.net/publication/ 228636479_In_vitro_models_for_antioxidant_activity_evaluation_and_some_medicinal_plants_possessing_antioxidant_properties_An_overview
4. Alsahafi M, Cramer P, Chatur N, Donnellan F. (2017).The effect of Prucalopride on small bowel transit time in hospitalized patients undergoing capsule endoscopy. Canadian Journal of Gastroenterology and Hepatology , Article ID 2696947, 7 pages. doi: 10.1155/2017/2696947
5. Sajid MS, Hebbar M, Baig MK, Li A, Philipose Z. (2016). Use of Prucalopride for chronic constipation: A systematic review and meta-analysis of published randomized controlled trials. J Neurogastroenterol Motil, 22 (3),412-22. DOI:10.5056/jnm16004
6. Kajiya M, Silva MJ, Sato K, Ouhara K, Kawai T.(2009). Hydrogen mediates suppression of colon inflammation induced by dextran sodium sulfate. Biochemical and Biophysical Research Communications, 386(1),11-15. DOI:10.1016/j.bbrc.2009.05.117
7. Ganapaty S, Chandrashekhar VM, Chitme HR, Narsu ML.(2007). Free radical scavenging activity of gossypin and nevadensin: An in-vitro evaluation. Indian J Pharmacol, 39(6):281-83. DOI: 10.4103/0253-7613.39147
8. Kim PKM, Zamora R, Petrosko P, Billiar TR. (2001). The regulatory role of nitric oxide in apoptosis. Int Immunopharmacol,1(8),1421–41. URL:https://www.ncbi.nlm.nih.gov/pubmed/11515809