

SHORT COMMUNICATION

EFFECT OF AQUEOUS EXTRACT OF *BRASSICA OLERACEAE* VAR *ITALICA* (BROCCOLI) INFLORESCENCE IN PHENYLHYDRAZINE INDUCED ANEMIC RATS

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The study was designed to evaluate the antianemic activity of aqueous extract of *Brassica oleraceae* var *italica* (Broccoli) inflorescence in phenylhydrazine induced anemia in Sprague Dawley rats. Phenylhydrazine treatment significantly decreased haemoglobin, RBC and MCH compared to normal group indicating the development of anemia. Standard group which received ferrous sulphate prevented all the changes caused by phenylhydrazine. Both the test groups showed significant improvement in RBC, haemoglobin and MCH. Phenylhydrazine treatment significantly increased WBC because of immune response and test groups normalized WBC. The present study concluded that aqueous extract of *Brassica oleraceae* var *italica* inflorescence has antianemic potential.

Key words: Anemia, *Brassica oleraceae*, Haemoglobin, RBC, Phenylhydrazine, Broccoli.

INTRODUCTION

Anemia is indicated as the decrease in the number of erythrocytes or the oxygen carrying capacity of blood which does not meet the physiological demand. Anemia is caused due to various factors such as inadequate absorption or intake of iron, reduced intake of vitamin B₁₂ or folic acid, destruction of red bone marrow, hereditary conditions etc. (Koffuor *et al* 2012). Around 1.62 billion people are affected by anemia in the world which calibrates to 24.8% of the total population. Anemia is mostly prevalent in under developed and developing countries where malnutrition is a major problem. The present day therapy is based on herbal formulations rather than synthetic preparation for their low cost and easy availability.

Plants are well known for their metabolites responsible for their biological effects since ancient times (Deb *et al* 2013; Rashid *et al* 2014; Sadanand and Palanivelu, 2015). *Brassica*

oleraceae var *italica* (Broccoli), belongs to family *Brassicaceae*, known as 'crown jewel of nutrition', is a rich source of minerals such as potassium, phosphorus, calcium, iron, zinc, selenium and sodium, vitamins, especially vitamin A, C, K and folic acid (Madhu and Kochhar, 2014). The edible parts of broccoli are sprouts and florets commonly called as inflorescence (**Figure 1**).



Fig. 1. Broccoli plant with inflorescence

Brassica oleraceae var *italica* was reported to have anticancer, antioxidant (Gawlik-Dziki *et al* 2014), antiseptic (Sanchez-Moreno, 2002), antiulcer (Vasanthi *et al* 2009), hypoglycaemic activities (Park *et al* 2012). Traditionally, it has been used in anemia but there is no scientific proof to support this claim. Hence, the study was undertaken to evaluate the antianemic property of aqueous extract of *Brassica oleraceae* var *italica* inflorescences in phenylhydrazine induced anemia.

MATERIAL AND METHODS

Plant extract

Aqueous extract of *Brassica oleraceae* var *italica* (Broccoli) inflorescence was gift sample from Green Chem Bangalore.

Phytochemical screening of aqueous extract of *Brassica oleraceae* var *italica* inflorescence

Identification tests were performed to identify the presence of phytochemicals in aqueous extract of *Brassica oleraceae* var *italica* inflorescence (Evans, 2002).

Experimental animals

Sprague Dawley rats of either sex (200-250 g) were obtained from the animal house of Krupanidhi Pharmacy college, Bangalore, India approved by CPCSEA (378/01/ab/CPCSEA). All the animals were maintained in a well ventilated room and given access to feed and water *ad libitum*.

Experimental design

Animals were divided into 5 groups of six each. Group 1 served as control and received normal saline. For induction of anemia, phenylhydrazine 60 mg/kg, *i.p.* for was given for two days (Ebuehi and Mbara, 2011).

Group 2 received phenylhydrazine alone whereas other groups received treatment after induction of anemia. Group 3 served as standard and received Fefol® at dose 0.012 mg/kg,

p.o. (Chauhan *et al* 2014).

Group 4 and 5 received aqueous extracts of *Brassica oleraceae* var *italica* inflorescence at doses of 100 and 200 mg/kg, *p.o.* respectively (Patel and Sharma, 2014). Treatments with standard drug and broccoli extract were continued up to fourteen days.

Measurement of haematological parameters

On day fifteen, blood samples were withdrawn by retro orbital route under mild ether anaesthesia. Fresh blood was immediately collected into heparinised tubes for measurement of routine hematological parameters. Haemoglobin estimation was done using Sahli's method (Balasubramanian and Malathi, 1992). Red blood cell count and White blood cell count were done using Neubauer chamber method.

Mean Cell Haemoglobin (MCH) was calculated from following equation:

$$\text{MCH (pg)} = [\text{Hgb (g \%)} \times 10] / \text{RBC count (mil/mm}^3\text{)}$$

Statistical analysis

Statistical analysis was done by one-way ANOVA followed by Bonferroni multiple comparison test. All values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical investigation

Phytochemical investigation of aqueous extract of *Brassica oleraceae* var *italica* inflorescence showed the presence of alkaloids, saponins and terpenoids.

Effect on haematological parameters

Phenylhydrazine treatment significantly decreased haemoglobin, RBC ($p < 0.001$). There is no significant change in MCH, whereas WBC count is found to be elevated ($p < 0.001$) when compared with control (Table 1).

Table 1. Effect on haematological parameters on Hb, RBC, MCH, WBC

S. No.	Groups/Parameters	Hb gm% (gm/dl)	RBC ($10^6/\text{mm}^3$)	MCH (pg)	WBC ($10^3/\text{mm}^3$)
1	Control	15.28 ± 0.22	6.57 ± 0.50	23.78 ± 1.42	6.875 ± 0.39
2	Phenyl hydrazine	9.43 ± 0.15***	4.25 ± 0.28***	24.17 ± 2.20	10.15 ± 0.3***
3	Standard	15.02 ± 0.24###	6.26 ± 0.40##	24.5 ± 1.84	6.95 ± 0.37###
4	Low dose of Broccoli	14.20 ± 0.35###	5.86 ± 0.22#	24.25 ± 0.74	7.583 ± 0.43###
5	High dose of Broccoli	14.35 ± 0.27###	6.06 ± 0.13##	23.69 ± 0.89	7.050 ± 0.18###

Values are given as mean ± SEM, (n=6/group). ***p < 0.001 when compared with control. #p < 0.05 ##p < 0.01, ###p < 0.001 When compared with Phenyl hydrazine treated group.

Aqueous extract of Broccoli at both doses has shown increased haemoglobin ($p < 0.001$) when compared with phenylhydrazine treated group. Both low and high dose of Broccoli has increased RBC ($p < 0.05$ and $p < 0.01$) when compared with phenylhydrazine treated group. There was decrease of WBC count in standard and test groups ($p < 0.001$) when compared with phenylhydrazine treated group. The results obtained by high dose of broccoli treatment has similar potency to standard (**Table 1**). Phenylhydrazine treatment causes haemolytic anemia by formation of reactive oxygen species. Reactive oxygen species formed will cause oxidative damage to RBC. Free radicals can also increase aging process of RBC cells. As a result, there will be decrease in RBC and haemoglobin. WBC cells are elevated as a result of body's defence mechanism to get rid of haemolytic products (Koffuor *et al* 2012). Broccoli is a rich source of minerals like iron and vitamins like A, C, folic acid (Madhu and Kochhar, 2014) which may help in haemoglobin formation. The results of the present study confirmed the presence of alkaloids and saponins in aqueous extract of

Brassica oleraceae var *italica* inflorescence. Alkaloids are powerful antioxidants and prevent damage done to cells by free radicals (Ogbe *et al* 2010). Saponins help in enhancing natural resistance of body (Singh *et al* 1991). Hence, in the present study, alkaloids and saponins present in the plant may have protective role in phenylhydrazine induced anemia.

CONCLUSION

From the present study, it can be concluded that aqueous extract of *Brassica oleraceae* var *italica* inflorescence has antianemic activity and this may be attributed due to presence of various minerals, vitamins and secondary metabolites such as alkaloids and saponins. Further studies are required to know the exact mechanism.

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REFERENCES

- Balasubramaniam P, Malathi A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *J. Postgrad. Med.* 1992;38(1):8-9.
- Chauhan SP, Sheth NR, Suhagia BN. Haematinic evaluation of fruits of *Opuntia elatior* (mill) on mercuric chloride induced anemia in rats. *Int. J. Res. Ayurveda Pharm.* 2014; 5(1):115-22.
- Deb L, Bhattacharjee C, Shetty SR, Dutta A. Evaluation of anti-diabetic potential of the *Syzygium cuminii* (linn) skeels by reverse pharmacological approaches. *Bull. Pharm. Res.* 2013;3(3):135-45.
- Ebuehi OAT, Mbara KC. Biochemical studies of iron fortified gari fed to Phenyl hydrazine-induced anemic rats. *Am. J. Food. Technol.* 2011;6(6):472-482. [DOI: 10.3923/ajft.2011.472.482]
- Evans WC. Treas and Evans Pharmacognosy, 15th Edition, Elsevier (A Division of Reed Elsevier India Pvt. Limited): USA, 2002.
- Gawlik-Dziki U, Swieca M, Dziki D, Seczyk L, Zlotek U, Rozylo R, Kaszuba K, Ryszawy D, Czyz J. *BioMed Res. Int.* 2014;2014:1-14 (Article ID 608053).
- Koffuor GA, Sam GH, Dadzeasah PE, Owiafe EO, Asante-Gyaopong A. Erythropoietic effect of the ethanolic root bark extract of *Carissa edulis* in phenylhydrazine-induced anemic Sprague-Dawley rats. *Res. J. Pharmacol.* 2012; 6(2):20-4. [DOI: 10.3923/rjpharm.2012.20.24]
- Madhu, Kochhar A. Proximate composition, available carbohydrates, dietary fibre and anti-nutritional factors of Broccoli (*Brassica oleracea* L. var. *Italica plena*) leaf and floret powder. *Biosci. Discov.* 2014;5(1):45-9.
- Ogbe RJ, Adoga GI, Abu AH. Antianaemic potentials of some plants extracts on phenyl hydrazine induced anaemia in rabbits. *J. Med. Plants Res.* 2010;4(8):680-4. [DOI: 10.5897/JMPR09.487]
- Park J-H, Kim R-Y, Park E. Antidiabetic activity of fruits and vegetables commonly consumed in Korea: Inhibitory potential against α -glucosidase and insulin-like action *in vitro*. *Food Sci. Biotechnol.* 2012;21(4):1187-93. [DOI: 10.1007/s10068-012-0155-5]
- Patel V, Sharma V. Effect of *Brassica oleracea* extracts on blood glucose and antioxidant profile in streptozotocin induced diabetic rats. *J. Med. Pharm. Innov.* 2014;1(5):4-9. [DOI: 10.15272/2Fjmpi.v1i5.39]
- Rashid MMU, Hossain MR, Islam MN, Mostafa Kamal ATM, Yusuf ATM. Evaluation of cytotoxic and thrombolytic activities of methanolic extract of the flowers of *Sida acuta*. *Bull. Pharm. Res.* 2014;4(3):108-11.
- Sadanand VK, Palanivelu M. Investigation of the pharmacological activity of ethanolic extract of *Abrus precatorius* seeds. *Bull. Pharm. Res.* 2015;5(1):28-30.
- Sanchez-Moreno C. Review: methods to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol. Int.* 2002;8(3):121-37. [DOI: 10.1106/108201302026770]
- Vasanthi HR, Mukherjee S, Das DK. Potential health benefits of broccoli - a chemico-biological overview. *Mini Rev. Med. Chem.* 2009;9(6):749-59. [DOI: 10.2174/138955709788452685]
