Abstract:

The present study was focused to evaluate the anti-anaemic activity of *Mukia maderaspatana* (MM) and *Kedrostis foetidissima* (KF) of Cucurbitaceae family. Anaemia was induced by administration of phenylhydrazine in the dose of 10 mg/kg for 8 days. The rats with haemoglobin content less than 14 mg/dl were selected for the study. Then animals were divided into 7 groups of 6 animals each. Both extracts were given in the doses of 150 mg/kg and 300 mg/kg orally for 4 days. Blood samples were collected from the animals by sinus puncture on 1, 2, 3 and 4th week of the treatment. Blood samples were subjected to the estimation of red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb). Both the doses of MM and KF extracts significantly (P < 0.01) enhanced the RBC content from the initial week of treatment to 4th week of treatment when compared to that of standard drug (Vitamin B12). There was no significant change in the WBC content after the treatment of MM extract. But there was less significant (P < 0.05) decrease in WBC in Vitamin B12 and KF extract treated groups.

Keywords: Anti-anaemic; Albino rats; Phenylhydrazine; *Mukia maderaspatana*; *Kedrostis foetidissima*
Introduction

In vertebrates, anemia is a common hematological disorder associated with several conditions such as drug toxicity, parasites, genetic or acquired defects, and blood loss. Anaemia, a haemolytic affection, characterized by an insufficiency in quality and quantity of the red globules [1]. Hemoglobin is necessary for transporting oxygen to tissues and organs in the body. The reduction in oxygen available to organs and tissues when hemoglobin levels are low is responsible for many of the symptoms experienced by anemic people. The consequences of anemia include general body weakness, frequent tiredness, and lowered resistance to disease. Anemia can be a particularly serious problem for pregnant women, leading to premature delivery and low birth weight. It is of concern in children since anemia is associated with impaired mental and physical development. Anaemia is the common condition for the people of all developing countries because of lack of nutrition and frequent usage of drugs to treat diseases (haemolytic anaemia). The hemolytic activity of arylhydrazines, such as phenylhydrazine, dapsone hydroxylamine, divicine, may lead to acute hemolytic anemia in vertebrates [2]. Reactive oxygen species (ROS) is a collective term used for oxygen containing free radicals, depending on their reactivity and oxidizing ability. ROS participate in a variety of chemical reactions with bio-molecules leading to a pathological condition known as oxidative stress [3]. Under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into O$_2^-$ through mitochondrial respiration, phagocytosis, etc. ROS percentage increases during infections, exercise, exposure to pollutants, UV light, ionizing radiation, etc [4]. Antioxidants are employed to protect biomolecules from the damaging effects of such ROS.

Mukia maderaspatana (MM) and Kedrostis foetidissima (KF) are two plants of Cucurbitaceae family which are commonly used as recipes. K. foetidissima is a climber/prostate herb very effective in the treatment of asthma, chest pain and urinary tract infection [5], diarrhea, HIV [6], small pox and skin diseases [7]. M. madraspatana is a tendril climber/prostate herb. The plant was reported to have activities such as hepatoprotective [8], anti-rheumatic [9], diuretic, stomachic (a digestive tonic), gentle aperients, antipyretic and anti-flatulent, anti-asthmatic, anti-inflammatory, anti-diabetic and anti-bronchitis and is used for tooth ache besides its use in vertigo and biliousness [10,11]. Phytochemical investigation of both plants confirmed the presence of polyphenols, flavonoids, alkaloids, tannins and saponins [12,13].

This study aims at finding new affordable therapies, easily accessible, non expensive, able to treat anaemia and provide scientific evidence of the effectiveness of the traditional use of plants having anti-anaemic effect.

Materials and Methods

Plant material

The plants were collected from Tiruppur district of Tamilnadu, India. Authentications of both plants were done in botanical survey of India, Coimbatore.

Preparation of extract [14]

The dried parts of both plants were made into coarse powder and packed separately in soxhlet apparatus with 1:1 ratio of ethanol and water. The extraction was run for 36 h, then both extracts were dried and weighed. The % yield of hydroalcoholic extract of M. madraspatana (HAEMM) was 22.6 g and hydroalcoholic extract of K. foetidissima (HAEKF) was 30.7 g.

Animals

Wistar rats weighing between 150-220 g (both sex) were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. All animals were allowed to free access to water and fed with standard commercial pellet rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines. Approval No: ECP/IAEC-A/2010/06/03.
**Phenylhydrazine induced anaemia** [15]

Animals were divided into seven groups of six animals each. Among 42 rats, 36 rats were made anaemic by the oral administration of phenylhydrazine (10 mg/kg) daily for 8 days and remaining 6 animals were served as normal control. Phenylhydrazine-treated rats with haemoglobin concentration < 14 g/dl were considered as anaemic and included for the study. The animals were grouped as follows:

- **Group I** - Normal control received 1 ml/kg of 0.5% CMC (carboxy methyl cellulose)
- **Group II** - Anaemic control received 1 ml/kg of 0.5% CMC (carboxy methyl cellulose)
- **Group III** - Treated with Vitamin B12 syrup (1 ml/kg)
- **Group IV** - Treated with HAEMM extract (150 mg/kg)
- **Group V** - Treated with HAEMM extract (300 mg/kg)
- **Group VI** - Treated with HAEKF extract (150 mg/kg)
- **Group VII** - Treated with HAEKF extract (300 mg/kg)

Blood samples were collected from the animals by sinus puncture on 1, 2, 3 and 4\textsuperscript{th} week of treatment. The blood samples were subjected to determination of red blood cells (RBC), white blood cells (WBC) and haemoglobin concentration (Hb).

**Statistical analysis**

Results were expressed as mean ± SEM (n=6). The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet’s test using GraphPad. P values < 0.05 or 0.01 were considered as significant.

**Result and Discussion**

Phenylhydrazine reduced the levels of RBC and Hb when compared to normal control. The HAEMM extract at 150 mg/kg did not show significant increase in Hb after 1\textsuperscript{st} week of treatment, but it enhances the Hb significantly (P < 0.05) after 2\textsuperscript{nd} week of treatment. Further treatment of HAEMM extract significantly (P < 0.01) raised the Hb level on 3\textsuperscript{rd} and 4\textsuperscript{th} week of treatment. At the same time HAEMM extract at dose of 300 mg/kg significantly (P < 0.01) elevated the Hb content after 1\textsuperscript{st} week of treatment itself. The HAEKF extract at 150 mg/kg and 300 mg/kg elevated the Hb content significantly (P < 0.01) from the initial day of treatment. The standard drug Vitamin B12 showed significant (P < 0.01) increase in Hb content after first week of treatment. The anti-anemic effect of HAEMM and HAEKF extract were equipotent as that of the standard drug Vit B12. The Tables 1-3 show the effect of HAEMM and HAEKF extracts on Hb, RBC and WBC concentrations in phenylhydrazine-induced anaemic rats.

Both the doses of HAEMM and HAEKF extracts significantly (P < 0.01) enhanced the RBC content from the initial week of treatment to 4\textsuperscript{th} week of treatment when compared with that of standard drug. There was no significant change in the WBC content after the treatment of HAEMM extract. But there was less significant (P < 0.05) decrease in WBC in Vit B12 and HAEKF extract treated groups.

It has been reported that phenylhydrazine causes oxidative damage to RBC by increasing the formation of reactive oxygen species [16,17]. Anaemia was observed after 6 days of injection. This seems to be recovery from anaemia in day 9 by the control group given only 0.5% of CMC, probably due to decreasing effect of the drug before a booster dose was given. Phenylhydrazine was reported to induce the development of Henz bodies on RBC membranes after six days of exposure, which protected them against further destruction by the chemical [18,19].

In this present study the RBC and Hb contents were increased due to recovery from free radical damage by the anti-oxidant character of MM and KF extracts [20,21]. WBC content was increased from 1\textsuperscript{st} to 3\textsuperscript{rd} week and then it was decreased because of phenylhydrazine-induced anemia is associated with immune activation and recovery [22]. Alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to the cells by free radicals or highly reactive oxygen species [23]. The differences in anti-anaemic potentials of the plant extracts might be due to the different phytochemicals present especially, the polyphenols (flavonoids).
Both plants in Cucurbitaceae have anti-anaemic activity in different potential. Further research has to be confirmed the role of phytoconstituents and their quantity.

References


