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Anti-anemic Effect of Momordica Cymbalaria Methanolic Extract on Phenylhydrazine Induced Experimental Rats

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KEYWORDS

Anemia, hematology, iron content, reticulocyte, bleeding, clotting.

ABSTRACT:

Introduction: The anti-anemic potential of *Momordica cymbalaria* fruits extract was assessed in phenyl hydrazine induced anemia in experimental animals by determining the hematology parameters, iron content, reticulocyte count, bleeding and clotting time, as well as the assessment of heart, liver and spleen weight in the experimental animals.

Methods: The rats administered with phenyl hydrazine exhibited significant reduction in the hematological parameters such as red blood cell count, hemoglobin level and hematocrit values and mean corpuscular volume (MCV) levels.

Results: On administration of *Momordica cymbalaria* fruits extract significantly increased the red blood cell count, hemoglobin level and hematocrit values and mean corpuscular volume (MCV) levels as compared to anemic control group. The phenyl hydrazine intoxicated animals significantly decreased the iron content and increased reticulocyte count in blood. The administration of different doses of *Momordica cymbalaria* fruits extract attenuated the iron content and reticulocyte count in blood. The clotting and bleeding time of blood was increased in animals treated with phenyl hydrazine. The administration of various doses of *Momordica cymbalaria* fruits significantly decreased the clotting and bleeding time. The marked increased in the weights of organs such as heart, liver and spleen were observed in animals treated with phenyl hydrazine. The significant reduction organs weights were observed in animals treated with different doses of *Momordica cymbalaria* fruits extract. The results obtained from the present study provides evidence of the anti-anemic potential of *Momordica cymbalaria* fruits extract against phenyl hydrazine induced anemia.

Conclusion: The observed anti-anemic activity of *Momordica cymbalaria* fruits extract may be due to the presence of phytochemicals such as flavonoids, alkaloids, steroids, saponins, iron content and vitamin C and its anti-oxidant activity could be the reason for anti anemic activity of the title plant.

1. Introduction

Anaemia is a condition in which the number of red blood cells or the concentration of haemoglobin in the body is lower than normal. Anaemia can occur at any age. WHO estimates that 42% of children under five and 40% of pregnant women worldwide suffer from anaemia. The prevalence of anaemia among women of reproductive age is 27.85% and among children under five years of age is 36.78% (World Health Organization, 2020). The main cause of anaemia is lack of nutrition (insufficient supply of iron), drug poisoning, blood loss, genetic or pathological diseases [1] and also several diseases can cause anaemia, such as Crohn's disease, chronic kidney disease, cancer, chronic inflammatory disease, gastrointestinal conditions, rheumatoid arthritis and more [2].

Medicines such as ferrous sulfate, iron dextran, folic acid, and deferoxamine are often used by people to prevent anemia. The occurrence of toxicity and

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addiction limits the therapeutic utility of these drugs. Given the inability of current therapies to manage the disease state, an alternative is clearly needed. People from different parts of the world use herbal medicines to alleviate affective disorders. Various herbs are used in complementary and alternative medicine to treat anemia. The potential use of safer and cheaper herbal medicines as anti-anemia agents has been reported because they can act against anemia without altering the physiological functions of the body [3].

Momordica cymbalaria belongs to the genus Momordica of the Cucurbitaceae family. It is commonly found in some parts of South India and Southeast Asian countries. However, it is used in traditional Indian systems of medicine. The fruit is high in calcium, iron, potassium, vitamin C and rich in fiber and is often used as a tonic, stomachic, stimulant and laxative to control skin diseases, rheumatism, ulcers, diarrhea and diabetic diseases in South Indian populations. In addition, plant extracts also have cardioprotective, hepatoprotective, nephroprotective and antioxidant, antibacterial activity [4].

In the traditional system of medicine, different parts of the plant are reported to be useful in different ailments. All parts of the plant and especially contains alkaloids, carbohydrates, flavonoids, saponins, steroids, triterpenes. *Momordica cymbalaria* fruits juice and leaf tea are used for diabetes, malaria, colic, cuts, infections, worms and parasites, as an emmenagogue and for measles, hepatitis and fever. Fruit pulp, leaf juice and seeds have anthelmintic activity. The root is astringent, abortifacient, aphrodisiac and is also used to treat constipation, indigestion, diabetes, diarrhea and rheumatism [5]. Upon extensive literature survey no research activity has been carried out on the title plant to evaluate the anti-anemic activity. Hence, the present study is under taken to evaluate the anti-anemic activity of methanolic extract of *Momordica cymbalaria* fruits in phenylhydrazine induced anemia.

2. Materials and methods

2.1 Collection of *Momordica cymbalaria* fruits

Momordica cymbalaria fruits were collected from farmers in Hubli after identification and authentication by Dr. Ramachandra Naik M, Professor & HOD, Dept. of Botany S.B Arts and KCP Science College, Vijayapur, Karnataka.

2.2 Preparation of extract:

The *Momordica cymbalaria* fruits were gently washed with tap water to remove adhering dust and soil particles. Then the fruits were shed dried and grinded to coarse powder. The powdered sample was extracted with methanol in Soxhlet apparatus. The excess solvent was then removed using rotary flash evaporator. The crude extract was stored in airtight container in refrigerator below 10^0 C for further work.

2.3 Preliminary phytochemical screening

The preliminary phytochemical investigation of *Momordica cymbalaria* fruits extract was carried out for the detection of various phytoconstituents by various procedures described in the previous research articles. [6-8]

2.4 Experimental animals:

The Wistar albino rats of 150 – 200g of either sex were used in the experimentation. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions i.e. Room temperature 27± 3^0 C, Relative humidity 65 ± 10, 12 hr. light/dark cycle. All the animals were fed with rodent pellet diet (VRK Nutritional Solutions, Pune, India) and water *ad libitium* under strict hygienic condition. Study protocol was approved from the Institutional Animal Ethical Committee (IAEC) before initiation of the experiment. [Ref.No. bldeacop/IAEC/2022/04].

2.5 Selection of different doses for the evaluation of anti-anemic activity [9]

Based on the previous literature survey, the extract did not cause any mortality of the experimental animals at a dose of 2000 mg/kg. Hence, 1/20th, 1/8th and 1/4thLD 50 cutoff values i.e. 100, 250 and 500mg/kg were used to assess the anti-anemic activity.

2.6 Evaluation of anti-anemic activity:

Anti-anemic activity of methanolic extract of *Momordica cymbalaria* fruits was evaluated against phenylhydrazine induced anemia in experimental rats.

Grouping

Male Wistar rats (150-200 g) were divided into five groups of six animals each.

Group 1:Non-anemic control, received vehicle only

Group 2: Anemic control, received Phenyl hydrazine (30 mg/kg) i.p. for first three days followed by vehicle for upto 21 days

Group 3: Phenylhydrazine (30 mg/kg) i.p. for first three days + MEMCF (100 mg/kg) p.o. upto 21 days

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Group 4:Phenylhydrazine (30 mg/kg) i.p. for first three days + MEMCF (250 mg/kg) p.o. upto 21 days
Group 5:Phenylhydrazine (30 mg/kg) i.p. for first three days + MEMCF (500mg/kg) p.o. upto 21 days
Later the blood sample was collected through the retro orbital sinus in an EDTA tube after the 24 hrs. last dose of drug administration for the determination of below mentioned parameters such as
Hematological Parameters [10]
Hemoglobin level
RBC count
Hematocrit
Mean corpuscular volume
Reticulocyte count [11]
Determination of total iron content [12]
Determination of bleeding and clotting time [13]
Further the rats were sacrificed and the weight of spleen, liver and heart was recorded.

2.7 Hematological parameter [14]

The 0.5 ml of collected blood sample was aspirated into digital cell counter and parameters like RBC, Hb, HCT and MCV were noted.

2.8 Enumeration of reticulocyte count [15]

2-3 drops of blood was taken in test tube to that 2-3 drops of staining solution was added (Staining solution is made up of using: 1 g Brilliant Cresyl Blue (BCB), 20 ml 3% tri sodium citrate and 80 ml 0.9% NaCl). Then the solution was mixed and kept aside at 37°C for 15-20 min in the water bath. Then smear was made on the slide and after drying the reticulocytes was examined using microscope under immersion oil. Than the numbers of reticulocytes were counted under the microscope and total number of reticulocytes were calculated using the formula

Formula:

$$\text{Reticulocyte count} = \frac{\text{Number of reticulocytes counted} \times 100}{\text{Number of RBC examined}}$$

2.9 Determination of Iron content [12]

0.5 ml of blood was transferred to a test tube containing 10 ml of concentrated HNO_3 (Nitric acid) followed by mixed and kept aside for one day. Then the sample was heated until the white smoke appeared, later five drops of $HClO_4$(Perchloric acid) was slowly added. Then the sample solution was cooled and filtered using filter paper and then diluted to 50 ml with distilled water in a volumetric flask. The absorbance was then recorded using spectrophotometer at 400 nm.

2.10 Determination of bleeding time [13]

The bleeding time was determined by using modified tail cutting method. Rats were placed inside a rat restrainer apparatus (plastic cylinder with multiple openings), by which the tail to extend outside. The rats were kept at room temperature throughout the procedure. Bleeding time was determined by making a 2 mm incision from the tail tip using a disposable surgical blade. Bleeding time was noted from the moment of appearance to until bleeding stops completely on the filter paper and expressed in seconds.

2.11 Determination of clotting time [13]

Blood was collected into a capillary tube from the retro orbital plexus. The stopwatch was started immediately then a small portion of capillary tube was broken at every 60 sec and the time taken to form thread-like structure was noted in seconds.

Further the rats were then sacrificed by over dosage of Ketamine for the removal of organs such as spleen, liver and heart to measure the weight of the organs.[15]

2.12 Statistical analysis

The data obtained from the above findings were subjected to statistical analysis using one-way ANOVA followed by Turkey Kramer Multiple Comparison Test to assess the statistical significance of the results.

3. Results

3.1 Preliminary phytochemical screening of *Momordica cymbalaria*fruits extract

The Preliminary phytochemical evaluation of *Momordica cymbalaria*fruits extract showed the presence of alkolides, flavonoids, carbohydrates, steroids and phenols.

3.2 Effect of *Momordica cymbalaria* fruits extract on hematological parameters

The rats administered with phenyl hydrazine exhibited significant reduction in the hematological parameters such as red blood cell count, hemoglobin level and hematocrit values and mean corpuscular volume (MCV) levels. On administration of *Momordica cymbalaria* fruits extract at different doses significantly increased the red blood cell count, hemoglobin level and hematocrit values and mean corpuscular volume (MCV) levels as compared to anemic control group. The results are tabulated in table 1.

3.3 Effect of *Momordica cymbalaria* fruits extract on Iron content in blood

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The phenyl hydrazine intoxicated animals exhibited decreased iron content in blood. The administration of different doses of *Momordica cymbalaria* fruits extract attenuated the iron content in blood as compared to anemic control group. The results are represented in table 2.

3.4 Effect of *Momordica cymbalaria* fruits extract on reticulocyte count in blood

The phenyl hydrazine induced anemia animals showed increased reticulocyte count in blood. The administration of various doses of *Momordica cymbalaria* fruits extract reversed the reticulocyte count in blood as compared to anemic control group animals. The results are indicated in table 3.

3.5 Effect of *Momordica cymbalaria* fruits extract on bleeding and clotting time

The clotting and bleeding time of blood was increased in animals treated with phenyl hydrazine in anemia control group animals. The administration of 100, 250 and 500 mg/kg doses of *Momordica cymbalaria* fruits significantly decreased the clotting and bleeding time as compared to anemia control animals. The results are tabulated in table 4.

3.6 Effect of *Momordica cymbalaria* fruits extract on organ weights

The marked increased in the weights of organs such as heart, liver and spleen were observed in animals treated with phenyl hydrazine. The significant reduction in the organs weight were observed in animals treated with different doses of *Momordica cymbalaria* fruits extract. The results are tabulated in table 5.

4. Discussion

Anemia is a condition in which the number of red blood cells or the concentration of hemoglobin in the body is lower than normal. Anemia can occur at any age. WHO estimates that 42% of children under five and 40% of pregnant women worldwide suffer from anaemia[1].

The anti-anemic activity of any drug or herbal extract are measured by various experimental animal models. Among which the PHZ-induced anemia model in experimental models is widely used because of rapid and reliable. the experimental animals such as rats, were commonly used animals because they provide the info about disease, genetics, effect of drugs.[17]

PHZ is a non-immunogenic chemical and strong oxidant which extensively used in the pharmaceutical industry. The PHZ produces several toxic effects includes hemolytic anemia, hypoxia, inflammation, variation in the liver, kidney, and heat. In the case of hemolytic anemia, the PHZ decrease the life span of RBC and increased tissue iron absorption. The oxidation of PHZ leads to the generation of reactive oxygen species (ROS), free radicals, etc. The ROS and free radicals impaires RBC deformability, destabilization of Hb, MCV and HCT[18]. In the present study the administration of PHZ has been reported to cause a breakdown of RBC resulted in significant decreased RBC content, Hb level, MCV and HCT by non-immune mechanism. The MEMCF administration at different doses reversed the total RBC count, HB level and RBC related parameters such as MCV and HCT in experimental animals.

Reticulocyte counts are more sensitive than erythropoietin level. It is reported as percentage of total RBC count or absolute numbers[19]. During hemolysis, spleen became unable to deal with immature reticulocyte causing delay in reticulocyte maturation. So it is an important task for any drug or herbal extract to promote the maturation of reticulocytes. In the present study the positive effect of MEMCF was shown by significant decrease in the reticulocyte in PHZ treated rats.

The most reliable indication for decreased iron in anemia is low Hb content. The iron is very much essential for influencing the Hb production and also for formation of Heme molecules in the body[20]. The PHZ in control animals showed marked decrease in iron content. The treatment of MEMCF at different doses significantly increased the iron content in the blood in a dose dependent manner.

In PHZ induced anemia theoretically there is an impaired hemostasis which is due to damage in the blood vessels and affects its healing. The platelets are the blood cells which involved in coagulation[21] or may inhibit the formation of prostaglandins by the vessel wall during injury. Prostaglandin released during injury is responsible for vessel relaxation which leads to an increase in bleeding of blood in injury [22]. The administration of various doses of MEMCF produced significant reduction in the clotting and bleeding time suggest that the MEMCF have positive effect on the hemostatic phase of wound healing, may act on integrity of blood vessel or involvement of platelets forming a hemostatic plug.

The anemia caused by PHZ causes the stimulation erythropoiesis resulting in splenomegaly, hepatomegaly [23] and cardiomegaly. Which corroborate in the present study with significant increase in the weight of spleen, liver and heart in PHZ treated animals. the administration of various doses of MEMCF significantly decreased the spleen, liver and heart weight thus provides the protective effect against inflammatory changes in the spleen, liver and heart.

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The phytochemicals such as flavonoids, phenolic acid, etc are the source of natural antioxidants present in medicinal plants. The flavonoids have shown to improve the hematological parameters, increased in the iron content [24]. In addition to this the secondary metabolites includes saponins, glycosides might be also responsible for anti-anemic potential of medicinal plants used in the traditional system of medicine [25]
In the present study MEMCF exhibited the presence of phytochemicals such as flavonoids, alkaloids, steroids, saponins [26], iron content [8] and vitamin C[27] and its anti-oxidant[28] activity could be the reason for anti-anemic activity of the title plant.

5. Conclusion

The results obtained from the present study provides evidence of the anti-anemic potential of *Momordica cymbalaria* fruits extract against phenyl hydrazine induced anemia. The observed anti-anemic activity of *Momordica cymbalaria* fruits extract may be due to the presence of phytochemicals such as flavonoids, alkaloids, steroids, saponins, iron content and vitamin C and its anti-oxidant activity could be the reason for anti-anemic activity of the title plant.

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Table 1: Effect of MEMCF on Hematological parameters

Group	Hematological Parameters			
	RBC ($10^6/\mu l$)	**Hb (g/dL)**	**HCT (%)**	**MCV (fL)**
Normal control	5.16±0.25	32.1±0.46	29.25±0.98	62.4±1.9
Anemia control	1.83 ± 0.11$^{@}$	10.3 ± 0.56$^{@}$	11.11 ± 0.66$^{@}$	61.88 ± 0.40$^{@}$
MEMCF (100 mg/kg)	2.89±0.26*	22.1±0.81***	18.05±1.86***	56.36±0.51***
MEMCF (250 mg/kg)	3.9±0.13***	29.65±0.52***	22.91±0.49***	57.8±0.34*
MEMCF (500 mg/kg)	4.77±0.26***	32.6±0.46***	28.4±0.99***	62.8±2.2*

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Values are expressed as Mean ± SEM, (n=6). @p< 0.001, * p< 0.05, ** p< 0.01, *** p< 0.001 as compared to Anemia control.

Table 2: Effect of MEMCF on Iron content

Group	Iron content
Normal control	0.396 ± 0.01
Anemia control	0.336 ± 0.01@
MEMCF (100 mg/kg)	0.340 ± 0.04*
MEMCF (250 mg/kg)	0.357 ± 0.05*
MEMCF (500 mg/kg)	0.392 ± 0.01*

Values are expressed as Mean ± SEM, (n=6). @p< 0.001, * p< 0.05, ** p< 0.01, *** p< 0.001 as compared to Anemia control.

Table 3: Effect of MEMCF on reticulocyte count

Group (n=6)	Reticulocyte count
Normal control	4.58 ± 0.07
Anemia control	7.21 ± 0.04@
MEMCF (100 mg/kg)	6.8 ± 0.05***
MEMCF (250 mg/kg)	6.2 ± 0.05***
MEMCF (500 mg/kg)	5.2 ± 0.04***

Values are expressed as Mean ± SEM, (n=6). @p< 0.001, * p< 0.05, ** p< 0.01, *** p< 0.001 as compared to Anemia control.

Table 4: Effect of MEMCF on Bleeding and Clotting time

Group	Bleeding time	Clotting time
Normal control	110 ± 6.32	55 ± 5
Anemia control	200 ± 6.32@	140 ±6.32@
MEMCF (100 mg/kg)	170 ± 6.32*	115 ± 5*
MEMCF (250 mg/kg)	145 ± 5**	85 ± 5**
MEMCF (500 mg/kg)	115 ± 5***	50 ± 6.32***

Values are expressed as Mean ± SEM, (n=6). @p< 0.001, * p< 0.05, ** p< 0.01, *** p< 0.001 as compared to Anemia control.

Table 5: Effect of MEMCF on organ weight

Group	Organ weight		
	Heart	liver	Spleen
Normal control	0.63 ± 0.02	4.23 ± 0.09	0.26 ± 0.01
Anemia control	0.73 ± 0.01@	5.56 ± 0.08@	0.45 ± 0.01@
MEMCF(100 mg/kg)	0.69 ± 0.01*	4.96 ± 0.04***	0.38 ± 0.01***
MEMCF(250 mg/kg)	0.63 ± 0.01***	4.55 ± 0.06***	0.33 ± 0.01***
MEMCF(500 mg/kg)	0.57 ± 0.01***	4.33 ± 0.02***	0.28 ± 0.007***

Values are expressed as Mean ± SEM, (n=6). @p< 0.001, * p< 0.05, ** p< 0.01, *** p< 0.001 as compared to Anemia control.