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Pharmacological Evaluation of Amarathus blitum L Aqueous Extract on Adenine Induced CRF Model of Anemia and Muscle Coordination in Rats

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KEYWORDS

Chronic Renal Failure, muscle coordination, Amaranthus, anemia, hematological parameters.

ABSTRACT:

Introduction: Amaranthus blitum L, (family: Amaranthaceae), is commonly known as spiny amaranth. It is the traditionally used plant as antipyretic, appetizer, diuretic, febrifuge, haematinic and laxative. This study investigated the impact of Amaranthus blitum L aqueous extract (ABLAE) on adenine induced Chronic Renal Failure (CRF) model of anemia to assess the extract's potential therapeutic benefits.

Objectives: Primary objective of this research is to evaluate impact of Amaranthus blitum L aqueous extract (ABLAE) on chronic renal failure induced anemia, using an adenine induced anemia model. Additionally, to explore the effect of extract on muscular coordination.

Methods: Rats exposed to adenine were treated with Amaranthus blitum L aqueous extract, at doses 200mg/kg, 400mg/kg and the hematological parameters along with biochemical parameters were evaluated in adenine induced CRF model of anemia. Additionally effect of Amaranthus blitum L aqueous extract on muscle coordination were determined by Rota rod test and grip strength measurement.

Results: Results indicate that Amaranthus blitum L aqueous extract (ABLAE), dose dependently increases hematological parameters like RBC count, hemoglobin level and hematocrit percentage along with erythropoietin and transferrin level in adenine induced CRF model of anemia. ABLAE dose dependently increases duration of time that rats spent on the rotating rod in Rota rod test and enhances grip strength, which indicates muscle strengthening effect.

Conclusions: Amaranthus blitum L aqueous extract at doses 200mg/kg, 400mg/kg demonstrates promising anti-anemic potential in adenine-induced CRF model of anemia in rats and improves muscle strength. Further research and development as a novel treatment for chronic kidney diseases induced anemia and muscle coordination at molecular level is required.

1. Introduction

Anemia is characterized by decrease number of red blood cells, low hemoglobin and hematocrit. World's one third people are affected by this condition. Anemia impairs an individual's ability to work effectively and productively along with neurological development [1]. Because of lack of oxygen, it can lead to symptoms like weakness, tiredness, less work efficiency, or difficulty in concentrating [2]. In addition, it reduces body's exercise capacity [3] and overall physical performance [4]. Kidney is multifaceted organ with numerous crucial functions. Among them is the production of hormone which is required for maintaining hematological balance [5]. Erythropoietin hormone which is synthesized by kidney is necessary for production of red blood cells. Renal insufficiency results in deficiency of erythropoietin, leading to anemia development [6]. Chronic kidney disease (CKD) is associated with anemia and is linked to reduced quality of life, higher cardiovascular disease (CVD) risk, increased rate of hospitalizations, cognitive decline, and higher mortality rates [7].

Amaranthus blitum L, (family: Amaranthaceae), is commonly known as spiny amaranth. It is the traditionally used plant as antipyretic, appetizer, diuretic, febrifuge, haematinic and laxative [8]. It is

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reported to contain antioxidant constituents, which have therapeutic benefits in many disease conditions like diseases of the cardiovascular system, neurodegenerative disease, atherosclerosis, cancer, cataracts, retinopathy, arthritis and emphysema [9].

2. Objectives

Primary objective of this research is to evaluate impact of Amaranthus blitum L aqueous extract (ABLAE) on adenine induced chronic renal failure anemia model. Additionally, to explore the effect of extract on muscular coordination.

3. Methods

2.1 Drugs and chemicals

Adenine, ether, EPO ELISA kit, transferrin ELISA assay kit, Oxymetholone.

2.2 Plant material and authentication

Fresh leaves of Amaranthus blitum L were collected from the farm of Yadrav, Ichalkaranji, Kolhapur District, Maharashtra, India. A plant specimen was submitted for authentification and it was verified by Botanist, Dr. Vikas B. Awale, from Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya, Sangliwadi, Sangli, Maharashtra.

2.3 Extract preparation

Amaranthus blitum L (ABL) fresh leaves were washed and shed dried under room temperature for the period of two weeks. The dried leaves were powdered and macerated with water. Amaranthus blitum L Aqueous Extract (ABLAE) was stored in refrigerator for further experimental use. The percentage yield of the Amaranthus blitum L Aqueous Extract (ABLAE) was calculated.

2.4 Phytochemical screening

Amaranthus blitum L Aqueous Extract (ABLAE) were subjected to the physicochemical tests to identify presence of alkaloids, glycosides, tannins, saponins, triterpenoids, flavonoids, and phenolics. [10].

2.5 Pharmacological evaluation

2.5.1 Approval of research protocol

All the experimental protocols were reviewed by the Institutional Animal Ethical Committee (IAEC) constituted as per guidelines of the CPCSEA (Committee for Purpose of Control and Supervision of Experimental Animals), India [11].

2.5.2 Experimental Animals

Female Wistar rats weighing between 200gm ± 20% and age between 8-12 weeks were chosen for the experimental study. They were housed in spacious cages provided with standard diet, and continuous access to food and water throughout the study. These cages were placed in a laboratory maintained at temperature 240C ± 10C, relative humidity 45-55%, and 12 hour light: 12 hour dark cycle. Food was withdrawn for 3 hr prior to the initiation of test.

2.5.3 Acute toxicity study

The acute oral toxicity study of the Amaranthus blitum L Aqueous Extract (ABLAE) was carried out in accordance with the Organization for Economic Co-operation and Development (OECD) 420 guideline with a maximum single dose 2000 mg/kg. Six female Wistar rat with weight ranging between 200gm ± 20% and age between 8-12 weeks were chosen for the study. The maximum single dose was administered by oral gastric intubation. After administration of the dose, each animal was then observed for 30 minutes, intermittently for the next 24 hr, and subsequently, every day for a total duration of 14 days. Signs of toxicity and/or mortality were monitored throughout this observation period [12, 13].

2.5.4 Adenine-induced CRF model of anemia

Five groups of female Wistar rats were randomly chosen and subjected to a four week treatment as shown in Table 1. Throughout the study period, all groups had access to both feed and water ad libitum. 24 hours after completing the treatment, the each rat was anesthetized using mild pet ether anesthesia. Blood samples from these anesthetized rats were collected through retro-orbital route and preserved in tubes coated with EDTA. Hematological and biochemical analysis were conducted on the collected blood samples [14].

Table 1: Adenine induced CRF model of anaemia (n=6)

Sr No.	Name of Group	Treatment	Oral Doses, frequency	Blood withdrawal
1	- Ve Control	Vehicle	0.5ml, daily	Retro orbital sinus, 0.5-1ml on 1st, 9th, 18th and 28th day using Pet. Ether.
2	+ Ve Control	Vehicle + adenine	0.75 %, w/w, in feed	
3	Standard	Orofer-XT+ adenine - 0.75 %, w/w, in	10mg/kg, daily + 0.75 %, w/w, in feed	

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		feed		
4	ABLAE 200mg/kg	ABLAE + adenine - 0.75 %, w/w, in feed	200 mg/Kg, once daily + 0.75 %, w/w, in feed	
5	ABLAE 400mg/kg	ABLAE + adenine - 0.75 %, w/w, in feed	400 mg/Kg, once daily + 0.75 %, w/w, in feed	

2.5.5 Erythropoietin (EPO) assay

Serum EPO level was evaluated using rat EPO ELISA kit (Krishgen Biosystems) following the procedure given by the manufacturer. The absorbance will be recorded at 450 nm using ELISA microplate reader [15].

2.5.6 Quantification of transferrin content

The transferrin content in plasma of treatment and control group animals were estimated by the transferrin ELISA assay kit. (Krishgen Biosystems) The plasma will be separated and analyzed for the transferrin content by ELISA kit protocol. Samples, standards, and reagents will be prepared according to the protocol [15].

2.5.7 Rota rod test

On day 1, animals were placed on the rotating rod with the adjusted speed of 20 rotations/min for 300 seconds. The two trials were conducted and the animals staying on the rotating rod for 300 seconds during these successive two trials were selected for this study. Animals were grouped into – 1. Control group: received Normal saline solution, 2. Standard group: received standard drug Oxymetholone (Anadrol) 50mg/kg and 3. Two test groups: received ABLAE at dose of 200mg/kg and 400mg/kg respectively for seven days. On seventh day, 45min after the treatment, rats placed on a rod rotating at speed of 20 rotations/ min. The duration of staying on the rod of the individual animal were recorded with cut off time of 300 sec. [16].

2.5.8 Grip-strength measurement

Kondziela's inverted screen method was used to measure grip strength. Animals were grouped into 1.Control group received Normal saline solution, 2. Standard group received standard drug Oxymetholone (Anadrol) 50mg/kg and 3. Two test group received ABLAE at dose of 200mg/kg and 400mg/kg respectively for seven days. On day 1 and day 14 after ABLAE treatment, animals were placed on wire mesh screen and screen was inverted. The time when animal releases their hind limb and falls off is being recorded [17].

4. Results

The experimental results were expressed as mean ± standard deviation (SD). All the data were analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using the statistics software Prism graph pad.

The preliminary qualitative phytochemical analysis of the ABLAE showed presence of flavonoids, glycosides, phenolics, tannins and triterpenoids . The percentage yield of the extract was 11.8%.

3.1 Anti-anemic activity

Experimental protocol was approved by IAEC of Biocyte Institute of Research and Development, Sangli. (IAEC/Sangli/2022-23/01).

3.2 Acute toxicity study

No signs of toxicity/mortality were observed during the two week study. As the doses used in the later study were 5-10 times smaller than the fixed dose used in the acute toxicity study, we can believe that later study was conducted with safe doses.

3.3 Adenine-induced CRF model of anemia

In adenine induced CRF model, ABLAE group, 400mg/kg group and standard group (Orofer XT 10mg) along with concurrent administration of adenine exhibited significant activity ($P < 0.01$) in determination of hematological parameters as compared to the positive control group. [Table 2]

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Table 2: Effect of Amaranthus blitum L Aqueous Extract (ABLAE) on Red blood cell count, Hemoglobin level and Hematocrit % in Adenine induced CRF model of anemia.

Group	RBC count (lacs/mm3)				Hemoglobin count g/dl				HCT count %			
	Day 1	Day 9	Day 18	Day 28	Day 1	Day 9	Day 18	Day 28	Day 1	Day 9	Day 18	Day 28
Negative control	65.6 ±0.50	61.9 ±0.45	61.0 ±0.21	65.8 ±0.31	12.34 ±0.24	12.68 ±0.12	12.54 ±0.56	12.17 ±0.42	43.01 ±1.21	42.43 ±1.43	41.85 ±2.13	41.45 ±1.54
Positive control	61.3 ±0.43	40.3 ±0.87	42.3 ±0.35	46.8 ±0.43	13.12 ±0.46	8.54 ±0.18	8.67 ±0.34	9.56 ±0.15	41.91 ±2.15	26.42 ±1.74	28.14 ±1.56	31.32 ±1.45
Standard	68.0 ±0.67	38.7 ±0.46	56.7 ±0.53	65.4 ±0.46**	12.15 ±0.25	8.24 ±0.21	11.56 ±0.15	13.24 ±0.18 ***	41.32 ±1.43	26.65 ±1.65	33.46 ±1.33	41.12 ±1.78**
ABLAE 200mg/kg	62.5 ±0.24	36.5 ±0.76	45.6 ±0.63	65.6 ±0.34**	12.84 ±0.33	7.25 ±0.56	11.14 ±0.37	12.76 ±0.26 *	41.45 ±2.01	24.54 ±2.25	32.54 ±1.55	39.52 ±1.55*
ABLAE 400mg/kg	65.6 ±0.47	36.5 ±0.32	53.2 ±0.43	75.2 ±0.23**	12.15 ±0.34	8.34 ±0.14	11.42 ±0.24	13.63 ±0.25 ***	40.67 ±1.56	25.45 ±2.61	35.34 ±1.56	41.14 ±1.96*

Values are expressed as mean ± SD followed by one-way ANOVA *P<0.05, ** P<0.01, ABLAE: Amaranthus blitum L Aqueous Extract, RBC: Red blood cell, HCT: Hematocrit.

3.4 Erythropoietin (EPO) assay & Quantification of transferrin (Tf) content

Daily administration of standard as well as ABLAE 200mg/kg and 400mg/kg along with concurrent administration of adenine resulted in a dose dependant improvement in erythropoietin (EPO)and transferring (Tf) levels compared with the negative control group.(Figure 1,2)

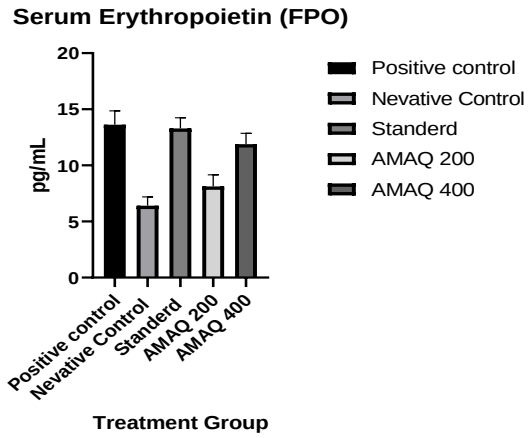

Figure 1.Effect of Amaranthus blitum L Aqueous Extract (ABLAE) on erythropoietin level in Adenine induced CRF model of anemia.

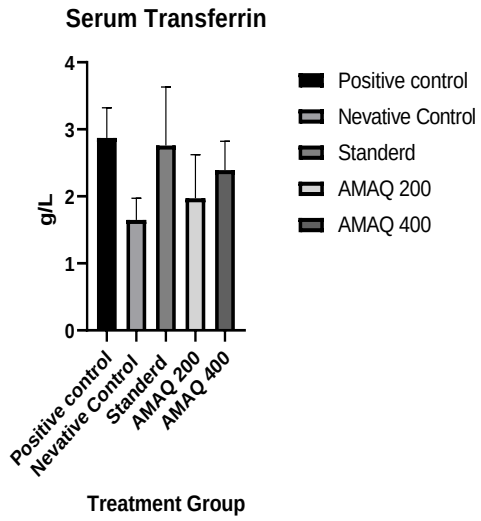

Figure 2. Effect of Amaranthus blitum L Aqueous Extract (ABLAE) on transferrin level in Adenine induced CRF model of anemia.

3.5 Rota rod test

During the Rota rod test, on seventh day both standard drug and ABLAE at dose 200mg/kg and 400mg/kg demonstrated significant increase in the time spent by the animals on rotating rod when compared to the control group. ABLAE at dose 400mg/kg spent more time on rotating rod, indicating maximum muscle strength. Results of Rota rod test showed that ABLAE significantly improved the performance in experimental animals (Table 3).

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Table 3: Effect of Amaranthus blitum L Aqueous Extract (ABLAE) on rat falling time in Rota rod test.

Group	**Rat falling time (Mean ± SEM)**
Normal control	56.83±12.01
Standard -Oxymetholone (Anadrol) 50mg/kg	65.47±20.19
ABLAE 200mg/kg	66.60±1.59*
ABLAE 400mg/kg	78±3.10**

All the values are expressed as mean ± SEM (n=6) followed by one-way ANOVA *P<0.05, ** P<0.01

3.6 Grip strength test

At the end of week seven, grip strength of the rats wereassessed both before (week 0) and after the administration of the ABLAE (200mg/kg & 400mg/kg). In grip strength measurement the time taken for release of the limb and drop down from the wire mesh in both standard and ABLAE (200mg/kg & 400mg/kg) group was significantly prolongedwhen compared with control group. (Table 4)

Table 4: Effect of Amaranthus blitum L Aqueous Extract (ABLAE) on drop down by grip strength measurement

a) Drop Down

Group	**Rat falling time before (Min)**	**Rat falling time after (Min)**
I. Normal control	2.4±0.4	2.3±0.2
II. Standard	3.1±0.2	3.14±0.4
III. ABLAE 200mg/kg	3.1±0.2	3.7±0.3*
IV. ABLAE 400mg/kg	3.3±0.4	4.5±0.1**

All the values are expressed as mean ± SEM (n=6) followed by one-way ANOVA *P<0.05, ** P<0.01

5. Discussion

In the present study, Adenine induced Chronic Renal Failure (CRF) model of anemia has been used. Adenine metabolizes to 2,8-dihydroxy adenine which forms crystal in the proximal tubular epithelia causing inflammation. In turn this inflammation leads totubule-interstitial fibrosis as well as anemia [18]. After birth glycoprotein hormone EPO is mainly synthesized in the kidney. Its production is severely reduced in patients with chronic kidney disease (CKD) with renal anemia, due to loss of cells producing erythropoietin [19]. In this study we found that in negative control group adenine feeding decreases red blood cell count, hemoglobin level, hematocrit percentage, erythropoietin and transferrin content in rat blood. However treatment with both standard and ABLAE (200mg/kg & 400mg/kg) dose dependently increases hematological and biochemical parameters. No previous research has evaluated the effect of ABLAE on biochemical parameters like EPO &Tf. Our results concerning these factors indicate positive outcomes might be because of protective influence of ABLAE on kidney tissues. ABLAE enhances kidney's ability to facilitate erythropoiesis process.

Endurance exercise leads to decreased level of blood components such as erythrocytes, ferritin, hematocrit and hemoglobin count. These changes often observed among athletes because of physical stress [20]. This impairs their physical performance, endurance and more susceptible to fatigue during prolonged exercise [21]. In this research ABLAE showed significant influence on models of muscle coordination. Administration of ABLAE at doses 200mg/kg and 400mg/kg led to an increase in the duration of time that rats spent on the rotating rod, suggesting improvement in motor coordination. Furthermore ABLAE administration showed positive influence on grip strength. Grip strength is a measure of muscular strengthening and endurance. Dose dependant enhancement in grip strength indicates muscle strengthening effect of ABLAE and its positive impact on muscle coordination.

Above findings and explanation showed that ABLAE at 200mg/kg & 400mg/kg doses has exhibited beneficial effect in adenine induced CRF model of anemia and demonstrated a positive effect on muscle coordination.

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