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EVALUATION OF ANTI-ANAEMIC AND ANTI-ALLERGIC ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF *ZIZIPHUS JUJUBA* FRUITS IN RODENTS

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ABSTRACT

The present study is an investigation of anti-anaemic and anti-allergic activity of aqueous and methanolic extracts of *Ziziphus jujuba* fruits. Anaemia was induced by the administration of phenylhydrazine. Oral administration of aqueous and methanolic extracts at two dose levels (200 mg/kg and 400 mg/kg) significantly enhanced the red blood cell count and hemoglobin concentration when compared to the anemic control rats. Similarly, both extracts were evaluated for anti-allergic activity against milk induced eosinophilia and leukocytosis. Administration of *Ziziphus jujuba* fruit extracts significantly inhibited the milk-induced eosinophilia and leukocytosis when compared with allergic control mice. Results obtained thus validate the traditional claim of the *Ziziphus jujuba* utilization in the treatment of anaemic and allergic conditions.

Keywords: *Ziziphus jujuba*, Anti-anaemic, Anti-allergic, Phenylhydrazine, Eosinophilia, Leucocytosis.

INTRODUCTION

Anaemia is a haematological condition with a quantitative deficiency of circulating haemoglobin. Anaemia can result from non-nutritional factors, such as haemorrhage, infections, chronic disease states or drug toxicity and from nutritional factors, including deficiencies of iron, certain vitamins, copper and protein and is irritating, uncomfortable and shortens the longevity of the individual if the condition is neglected [1, 2]. There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells [2, 3]. Anaemia is a major public health problem affecting a greater percentage of the world's population and is highly prevalent in developing regions of the world [4-6]. Management and treatment of this disorder with compounds or techniques (e.g. Hydroxyurea, Bone Marrow Transplantation and Blood Transfusion) are very expensive and out of reach of the masses and besides may expose the patient to mutagenicity, iron overload and other fatal risks [7].

Allergy is an immune disease origin which includes asthma, rhinitis, atopic eczema and dermatitis syndrome [8]. The burden of allergic diseases in India has been on an uprising trend in terms of prevalence as well as severity. Approximately 20% to 30 % of total population suffers from at least one of these allergic diseases in India. Allergy is a major problem for the 21st century, and this problem is predicted to worsen as this century moves forward [9]. There are countless number medications available in the market to reduce annoying allergic reactions. Anti-allergic drugs include cetirizine, chlorpheniramine maleate, decongestants, antihistamines, anti-inflammatory agents, anti leukotrienes and topical corticosteroids. However, the use of these drugs also suffers from adverse effects like sedation, dry mouth and immunosuppression. Anticholinergics and antihistamines

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are only effective for symptomatic relief, but fail to produce therapeutic and productive effect [10].

Medicinal plants have served through the ages as the mainstay in the treatment of diseases and preservation of human and animal health. *Zizyphus jujuba* Mill, called as Red date, Chinese date, and Bera (Pushto) belongs to family Rhamnaceae. Documented reports of jujuba fruits shown to produce anti-inflammatory, anti-obesity, immune-stimulating, antioxidant, gastrointestinal and hepatoprotective effect and inhibit foam cell formation in macrophages and jujuba containing herbal formulation found to exhibit anticancer activity [11]. Hence, this study aims at finding new affordable therapies, easily accessible, non expensive, able to treat anaemia and allergy using *Ziziphus jujuba* fruits.

MATERIAL AND METHODS

Plant collection and authentication

The specimen of the plant *Ziziphus jujuba* was collected locally and the sample of fruit was identified and authenticated by Prof. K. Madhava chetty, Reasearch Officer-Botany, Sri Venkateswara University, Tirupathi. A voucher specimen was made and deposited at the herbarium.

Preparation of aqueous and methanolic extracts of *Ziziphus jujuba*

Freshly collected fruits of the plant *Ziziphus jujuba* were washed, shade dried under room temperature, deseeded and cut into small pieces. The dried material was made to a coarse powder and About 1 kg of the powder was extracted with 2.5 L of methanol by cold maceration for 72 hours and filtered. The filtrate was dried in rotatory evaporator to obtain the methanol extract. Also 1 kg of the powder was extracted with distilled water (1000 ml) by cold maceration for 72 hours. The filtrate was freeze dried to obtain the aqueous extract. The extracts were subjected to phytochemical screening using standard methods [12].

Preliminary phytochemical analysis

Aqueous and methanolic extracts obtained by the above methods from *Ziziphus jujuba* fruits were subjected to qualitative test for the identification of various plant constituents by the standard procedures [12].

Experimental animals

Albino Wistar rats (150-200 g) and Swiss albino mice (20-25 g) of either sex were obtained from the animal house of Hindu College of Pharmacy, Guntur, Andhra Pradesh, used for the present study. The animals were housed in groups of six in polypropylene cages and maintained with constant temperature (20 ± 2°C) and humidity (50 ± 5%). Feed and drinking water were provided *ad libitum*. Healthy animals after the 1 week adaptation were used for the experiment and all procedures were performed in accordance with CPCSEA guidelines.

Evaluation of anti-anaemic activity

Phenylhydrazine induced anaemia

Anaemia was induced in rats by daily oral administration of phenylhydrazine (PHZ) at 10 mg/kg for 8 days. Rats that developed anaemia with haemoglobin concentration lower than 10 g/dl were recruited for the study [13, 14].

Grouping and treatment

The anaemic rats were randomly divided into seven groups (6 rats per group) and treated daily for 4 weeks as follows. The first group received distilled water (10 ml/kg). The second group is anaemic control. The group 3 animals received Vit B12 syrup (Becosules Syrup, Pfizer Ltd., India) (1 ml/rat). Animals in group 4 and 5 received the AEZJ at 200 and 400 mg/kg respectively. Animals in group 6 and 7 received the MEZJ at 200 and 400 mg/kg respectively. All administrations were by oral intubation.

Analysis of haematological parameters

Blood was collected by ocular puncture after overnight fast. The blood was collected before induction of anaemia, after induction of anaemia with PHZ and during 0, 1, 2, 3 and 4 weeks of treatments. The volume of blood collected (0.25 to 0.45 ml) did not affect blood parameters. The red blood cell count (RBC) and haemoglobin concentration (Hb) was determined by hematology Analyzer RX-50V.

Evaluation of Anti-allergic activity

Milk-induced Leucocytosis and Eosinophilia

Swiss albino mice (20-25 g) were randomly divided into seven groups (6/group). Group-1 received distilled water (10 ml/kg, p.o.), group 2 administered with boiled and cooled milk (4 ml/kg, subcutaneously), group 3 received dexamethasone (0.27 mg/ kg, i.p.) group 4 and 5 received aqueous extract (200 and 400 mg/kg, p.o.) respectively, and group 6 and 7 received methanolic extract (200 and 400 mg/kg, p.o.) respectively. One hour later, treatment groups received boiled and cooled milk (4 ml/kg, s.c.). Blood samples were collected before and 24 h after milk administration from retroorbital plexus, under light ether anesthesia and full eosinophils and leukocytes were counted [15].

Statistical analysis

Results were expressed as mean ± SEM (n=6). The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad. P values < 0.05, 0.01 and 0.001 were considered as significant.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical analysis of

aqueous & methanolic extracts showed the presence of Carbohydrates, Saponins, Alkaloids, Proteins, flavonoids, Triterpenoids and Tannins.

Evaluation of Anti-anaemic Activity

Effect of aqueous and methanolic extracts of *Ziziphus jujuba* on the body weight.

Weight gain following treatment of anaemic rats with two different doses of aqueous and methanolic extracts of *Ziziphus jujuba* was significantly higher than those of anemic control group and these results were shown in table 1.

Effect of *Ziziphus jujuba* on red blood cells (RBC) and hemoglobin levels in phenylhydrazine induced anemic rats.

Oral administration of extracts of *Ziziphus jujuba* showed significant increase in red blood cell count and hemoglobin concentration, when the four tests groups was compared with anemic control. By the end of 4th week of treatment, higher doses (400 mg/kg) of aqueous and methanolic extracts showed more significant activity and these results were tabulated in table 2 and 3.

Evaluation of anti-allergic activity

Effect of *Ziziphus jujuba* on milk induced eosinophilia and leukocytosis

Subcutaneous administration of cow milk significantly elevated eosinophils, total WBC's and differential leukocytes (neutrophils and monocytes) in mice. Treatment with aqueous and methanolic extracts of *Ziziphus jujuba* (200 mg/kg and 400 mg/kg, p.o), prevented milk induced increase in eosinophils, monocytes, neutrophils and total WBC count and these results were tabulated in table 4.

Table 1. Effect of aqueous and methanolic extracts of *Ziziphus jujuba* on the body weight of experimental animals.

Treatment	Initial weight before inducement of anaemia (g)	Weight after induction of anaemia (g)	Weight after treatment with *Ziziphus jujuba* (g)	Weight gain/loss (g)
Normal control	148.8 ± 7.76	----	179.34 ±11.10	30.54
Anemic control	143.77 ± 16.60	134.50 ± 13.76	140.50±11.10	6
Vit B_{12} syrup (1 ml/kg)	I67.50 ± 8.95	153.04 ± 9.14	181.10 ± 5.97	28.06***
AEZJ (200 mg/kg)	164.30 ± 12.72	158.33± 7.50	170.83± 8.40	12.50**
AEZJ (400 mg/kg)	I67.33 ± 5.90	160.54± 10.66	184.04± 9.97	23.50***
MEZJ (200 mg/kg)	171 ± 9.72	155.54± 8.72	166.60± 4.73	11.10*
MEZJ (400 mg/kg)	168.5 ± 7.95	161.78± 5.33	182.58± 8.54	20.48***

Values are expressed in mean±SEM. *p<0.05, **p<0.01, ***p<0.001 compared with control group. n=6.

Table 2. Effect of *Ziziphus jujuba* on red blood cell (RBC) count in phenylhydrazine induced anemic rats

Treatment	Red blood cell (RBC) (x10^6/ µl)			
	1st Week	2nd Week	3rd Week	4th Week
Normal control	5.58±1.32	5.87±1.16	6.18±1.28	6.72±0.92
Anemic control	3.24± 0.11	3.17± 0.23	3.08± 0.31	3.05± 0.08
Vit B12 syrup (1 ml/kg)	5.06±0.16*	5.54 ±0.37*	6.06±0.16**	6.28±0.43**
AEZJ (200 mg/kg)	4.83 ± 0.23	4.97 ± 0.11*	5.17 ± 0. 32*	5.49 ± 0.48**
AEZJ (400 mg/kg)	5.04 ± 0.10*	5.32± 0.12*	5.87 ± 0.67*	6.14 ± 0.33***
MEZJ (200 mg/kg)	4.47 ± 0.17	4.77 ± 0.21*	5.03 ± 0.22*	5.23 ± 0.17*
MEZJ (400 mg/kg)	4.68± 0.17	4.83± 0.21*	5.11 ± 0.22*	5.77± 0.37**

Values are expressed in mean±SEM. *p< 0.05, **p< 0.01, ***p< 0.001 compared with control. n=6.

Table 3. Effect of *Ziziphus jujuba* on hemoglobin (Hb) levels in phenylhydrazine induced anemic rats

Treatment	Hemoglobin (gm/dl)			
	1st Week	2nd Week	3rd Week	4th Week
Normal control	12.92 ± 0.31	13.43 ± 0.21	13.25 ± 0.23	14.32 ± 0.29
Anemic control	9.93 ± 0.13	8.07 ± 0.19	8.18 ± 0.23	8.75 ± 0.27
Vit B12 syrup (1 ml/kg)	11.59 ± 0.21*	12.88 ± 0.45**	13.72 ± 0.49**	14.40 ± 0.46***
AEZJ (200 mg/kg)	10.85 ± 0.31	11.30 ± 0.24*	12.32 ± 0.17*	13.05 ± 0.39**
AEZJ (400 mg/kg)	11.28 ± 0.17*	11.88 ± 0.36**	12.88 ± 0.23**	14.12 ± 0.44**
MEZJ (200 mg/kg)	10.03 ± 0.41	10.80 ± 0.50	11.77 ± 0.32*	12.62 ± 0.32*
MEZJ (400 mg/kg)	11.13 ± 0.17	11.58 ± 0.18**	12.67 ± 0.45**	13.55 ± 0.28**

Values are expressed in mean±SEM. *p< 0.05, **p< 0.01, ***p< 0.001 compared with control group. n=6.

Table 4. Effect of aqueous and methanolic extract of *Zizyphus jujuba* on eosinophil, differential and total leukocyte count in mice

Treatment (mg/kg)	Eosinophils per cu. mm (×10c/µl)	Differential Leukocytes (×10^c/µl)		Total Leukocytes per cu.mm
		Neutrophils	Monocytes	
Normal control	58.54 ± 7.09	3.84 ± 0.24	44.4 ± 3.4	4827 ± 31.1
Allergic control	149.80 ± 11.66	12.60 ± 0.19	60.4 ± 6.8	9894 ± 40.8
Dexamethasone	64.23±5.44***	4.84±0.33***	45.44±4.5**	5437± 37.5***
AEZJ (200 mg/kg)	104.20 ± 13.17*	7.8 ± 0.90*	52.2 ± 4.2	8290 ± 51.6
AEZJ (400 mg/kg)	79.10 ± 8.74**	5.34 ± 0.36***	48.3 ± 5.4*	6687 ± 38.9**
MEZJ (200 mg/kg)	108. 54 ± 9.90*	8.31 ± 0.33*	54.3 ± 2.2	8530 ± 29.0
MEZJ (400 mg/kg)	89.40 ± 7.71**	6.32 ± 0.54**	49.8 ± 7.02*	7380 ± 34.1*

Values are expressed in mean±SEM. *p< 0.05, **p< 0.01, ***p< 0.001 compared with control group. n=6.

DISCUSSION

Phenylhydrazine (PHZ) is a well-known and effective hemolytic agent that is routinely used for inducing anemia in laboratory animals. PHZ has been categorized as a nonimmunologic drug whose effects are dose-related. It has been established that PHZ induces chemical changes in the red cell membrane and causes oxidative denaturation of hemoglobin, which results in precipitates of altered hemoglobin known as Heinz bodies that shorten erythrocyte life span [16]. In our present study, administration of phenyl hydrazine altered the function of RBC by haemolysis characterized by decrease in RBC and decrease in Hb concentration supporting the previous reports [17, 3].

Ziziphus jujuba fruit extracts significantly increased the concentration of haemoglobin and red blood cell count after four weeks of treatment. The increase in the blood indices was progressive giving the highest effect on the fourth week of treatment. The increase in the haematological indices exhibited by *Ziziphus jujuba* extracts might be connected with the phytoconstituents of the fruits such as flavonoids, vitamins, amino acids, organic acids, polysaccharides and microelements [18]. These constituents are well known haemopoietic factors that have direct influence on the production of blood and antioxidant properties inhibiting free radical induced damage to blood cells [19].

Eosinophil degranulation is an important immunological event responsible for allergic cutaneous inflammation manifested in cow's milk. Therefore increased eosinophils reflect the overall state of allergic condition. This is supported by the fact that asthma/allergy patients show increased eosinophilia and leukocytosis [20, 21] and can serve a sensitive cellular biomarker. The overall increase in eosinophils and leukocytes count following administration of milk may be due to the participation of bone marrow and T-cells derived lymphocytes [22]. In agreement with this, in our present study subcutaneous administration of cow milk significantly elevated eosinophil, total WBC's and differential leukocyte (neutrophils and monocytes) count in mice. Oral administration of aqueous and methanolic extracts of *Ziziphus jujuba* reduced cellular immune reactions (eosinophilia and leukocytosis count) induced by milk. Further, the anti-allergic potential of aqueous and methanolic extracts of *Ziziphus jujuba* was comparable with ethanolic extract reported previously [23]. Presence of phytoconstituents like flavonoids, triterpenoids, and saponins in jujuba fruits are known for their antioxidant property and also found to stabilize mast cell membrane and exhibit anti-allergic activity [24].

In conclusion, both aqueous and methanolic extracts of *Ziziphus jujuba* fruits exhibited potent anti-anemic and anti-allergic activities. Presence of several phytoconstituents alone or in combination could have attributed to the anti-anaemic and anti-allergic properties of *Ziziphus jujuba*. This result supports at least partially the traditional use of *Ziziphus jujuba* in the treatment of anaemia and allergy.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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