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### EVALUATION OF ERYTHROPOIETIC EFFECTS OF ETHANOLIC EXTRACT OF FRUIT OF PIPER CHABA IN ALBINO RABBITS

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**Abstract:** Since ancient civilizations plants and herbs have been used for treatment of different ailments. The presence of active ingredients in plants and herbs are responsible for production of therapeutic effect. The objective of this study is to investigate the erythropoietic effects of ethanolic extract of Piper chaba. Automated huma count plus was used for hematological examination of blood. Ethanolic extract of Piper chaba was suspended in DMSO and administered in dose of 150mg/kg after suitably adjusting with weight of rabbit. The dose was given once daily. The results showed increased in hemoglobin, erythrocyte count, hematocrit count, MCH level, MCHC level and decrease in MCV level. From above it is concluded that Piper chaba possesses pronounced erythropoietic activity so it can be beneficial in treatment of anemia.

**Keywords:** Anemia, Piper chaba, erythrocytes, hemoglobin and mean corpuscular volume.



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## INTRODUCTION

An integral part of traditional medicine is herbal medicine. World health organization has defined traditional medicine as diverse health knowledge, practices, beliefs and techniques which utilizes natural resources such as plants, animals, minerals, manual methods, meditation and spiritual exercises which are used alone or in combination to maintain well being as well as to diagnose, treat or prevent illness. [1]

Plants had been used for healthcare and medicinal purposes long before it was recorded in history. As early as 3000 BC Egyptian papyrus and ancient Chinese writing describe medicinal uses for plant. Herbal therapies were used in different traditional medical systems such as Traditional Chinese medicine and Ayurveda. Different herbs have been used in healing rituals by different cultures such as Native American and African. [2]

The use of herbs to treat different diseases is very common in under developed third world countries because it is cheaper and safer than modern allopathic medicine. According to survey conducted by World Health Organization 80% of the population of some African and Asian countries still prefer use of herbal medicine. [3]

The plant Piper chaba is an important species of genus Piper and belongs to family Piperaceae. The genus Piper comprises of 1000 or more species distributed commonly in the tropical and subtropical regions. The synonyms commonly used are Piper retrofractum, Piper officinarum and Piper maritime. The plant is commonly known as Java long pepper or choi. [4]

The plant Piper chaba grows yearly and is a lasting shrub. [5] Piper chaba is available in various parts of Singapore, Sri Lanka, Malaysia, Indonesia, India and other South Asian countries. It is also cultivated in Bangladesh in Khulna division. [4] The fruit oil of Piper chaba contains caryophyllene oxide,  $\beta$ -caryophyllene, few monoterpenes hydrocarbons, and high amount of aliphatic hydrocarbons and moderate content of sesquiterpenes. The content of caryophyllene oxide is 7.4% and that of  $\beta$ -caryophyllene is 39.7%. [6]

In short the whole Piper chaba plant contains the following constituents: 1-undecylenyl-3,4-methylenedioxy benzene, Alpha amyryl,  $\beta$  – sitosterol, [7]. Chavicine, Cineol, terpinan-4, 1- $\beta$  caryophyllene, Fructose, Glucose, Guineesine, lignan, [8] N-isobutyl-decatrans-2trans-4-dienamide, Palmitic acid, Pellitorine, [9] Piperidine, Piperocetadecalin, Piperine, Piperlonguminine, [10] Pipernonaline, Pipereicosalidine, Piplartine, Retrofractamide-D, Sesamin, Tetrahydropiperonic acid and Veneol. [6]

Piper chaba has been used in traditional medicine as carminative, stimulant, anti-hypertensive, muscle relaxant [11]. It is useful in liver diseases such as jaundice and for migraine headaches. It is a component of digestive and sleep inducing preparation [12].

The fruit of Piper chaba has shown a lot of potential in traditional medicine. It is used as an anti-flatulent, gastro-protective, appetizing property, as an expectorant, anti-tussive, anti-fungal agent. It also possesses cholesterol lowering properties [13]. The fruit is used as an anthelmintic, carminative, improves appetite and taste, useful in asthma, bronchitis, fever, inflammation, piles, pain in abdomen at anus. The fruit has stimulant properties and is used in hemorrhoidal conditions. [4]

The other activities exhibited by Piper chaba fruits are anti-inflammation, chemoprevention, hepatoprotection, antiangiogenesis, adipogenesis and immunomodulation.[14] In Ayurveda it has shown diuretic, anti-inflammatory, analgesic, antidiarrheal and CNS depressant activity in mice [15].

The aim of present study is to evaluate the erythropoietic effects of ethanolic extract of fruit of piper chaba on which so far no work has been done so it can be used in treatment of different hematological disorders especially anemia which is very common in third world countries.

#### **MATERIALS AND METHOD:**

##### *Collection of Plant material:*

The fruit of Piper chaba was provided by Dr. Iqbal Azhar from department of Pharmacognosy University of Karachi.

##### *Extraction of Plant Material:*

Inorder to reduce the microbial load the fruits were first washed with water. The fruits of Piper chaba were cut into small pieces and dried at 50°C, and then they were powdered and extracted. Next this powdered material was macerated with 95% ethanol for 3 days. It was then filtered and reduced to dryness under pressure. The process of maceration was repeated twice and then dried using evaporator. The percentage yield of Piper chaba was 10.6%.

##### *Selection of Animals:*

For hematological screening albino rabbits of either sex weighing 1000- 1600 grams were selected. Biochemical and hematological variations produced in rabbits and humans are comparatively similar therefore rabbits were selected for these tests. [16]

The rabbits were equally divided into 2 groups, each containing 10 animals. First group served as Control and second was given Piper chaba. Water and diet was given ad libitum for 7 days. The animals were kept in individual transparent cages in calm room under constant temperature of  $23 \pm 2^\circ$  C inorder to acclimatize them with the environment.

##### *Dosing regimen:*

For hematological testing rabbits were used. First group was taken as control and given DMSO (same calculated ml as other groups) orally. Second group was given Piper chaba 150mg/kg, the dose was adjusted based on weight of rabbits. Standard solution of 750mg/10ml was prepared in DMSO and by serial dilution method it was administered orally.

Animals were handled as per specifications provided in Helsinki Resolution 1964 and study was approved by our Board of Advanced studies and research vides Resol. NO.10 (6) dated 26-09-2012 & 16-10-2012.

##### *Hematological Testing:*

Before the testing sample collection is required.

##### *Sample Collection:*

7ml of blood samples were drawn from rabbits after 7 day dosing by cardiac puncture. For hematological study 2ml blood is taken in EDTA K3 tubes.

*Testing of Hematological parameters:*

For hematological examination i.e Red blood cell count, White blood cell count, Hemoglobin, and platelet count, in EDTA K3 tubes 2ml blood was collected [17]. These tests were conducted using automatic humacount (hematology analyzer model # 16400/S) (Human Germany). [17]

**RESULT:**

**Statistical Analysis:**

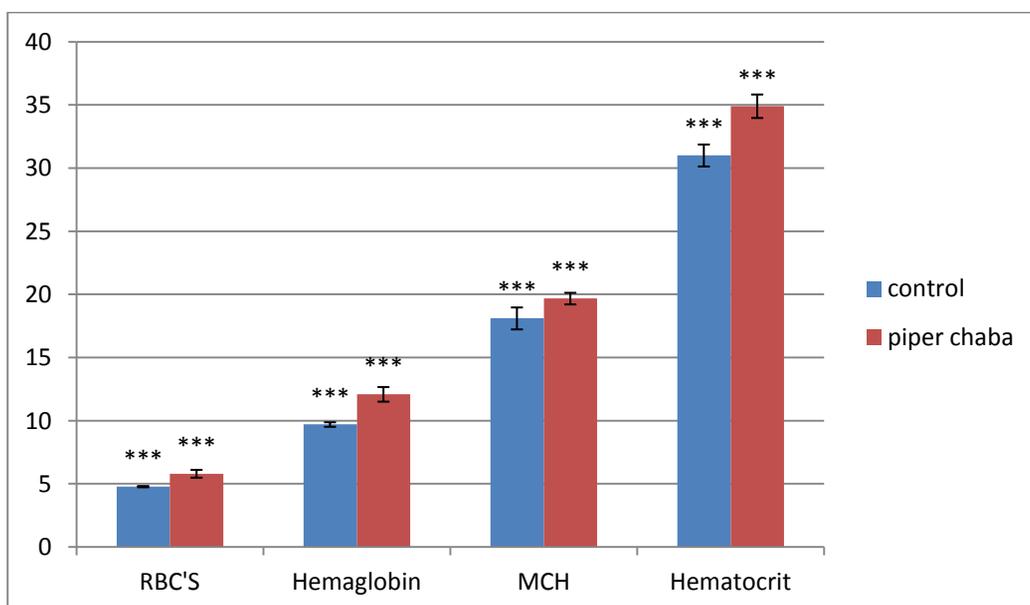
By taking mean of all the values they are compared with means of control and standard drug and by student significance t-test the significance of difference between means are determined. A value of  $p < 0.05$  is considered significant,  $p < 0.001$  as more significant and  $p < 0.0001$  as highly significant. By Alcarz and Jimenez method all statistical procedures are performed.[18]

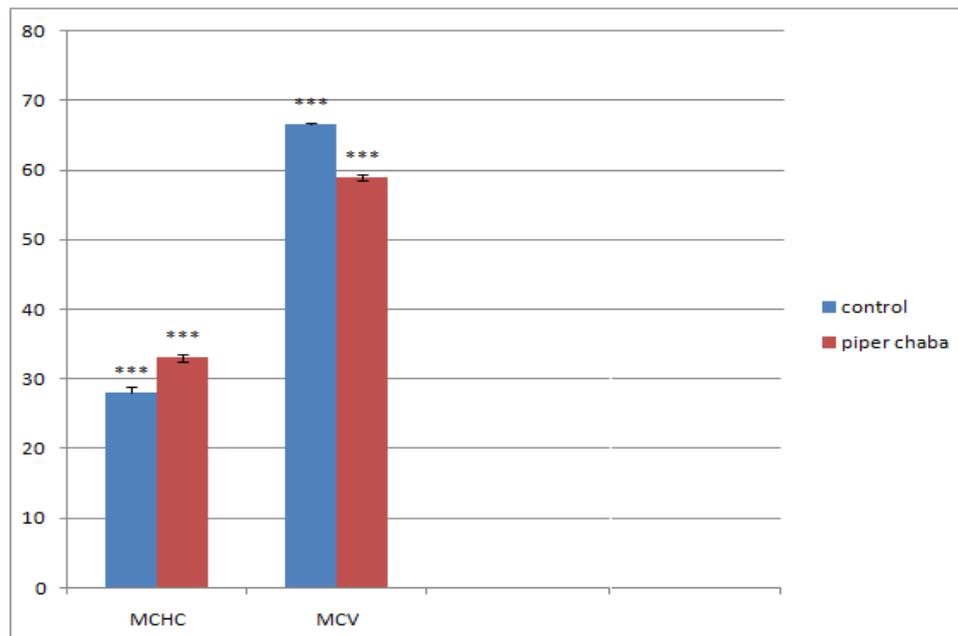
**Table: 1**

**Effect of Extract on Erythropoietic Parameters**

Parameters	Control Mean $\pm$ SD	Treated Mean $\pm$ SD	P value
Hemoglobin (g/dl)	9.7 $\pm$ 0.18	12.09 $\pm$ 0.58	***0.000
Hematocrit (%)	31.00 $\pm$ 0.87	34.9 $\pm$ 0.93	***0.000
RBC'S(million/ $\mu$ l)	4.78 $\pm$ 0.05	5.8 $\pm$ 0.31	***0.000
MCH (pg)	18.1 $\pm$ 0.87	19.67 $\pm$ 0.46	***0.000
MCV (fl)	66.57 $\pm$ 0.21	59.0 $\pm$ 0.42	***0.000
MCHC (g/dl)	28.0 $\pm$ 0.816	33.0 $\pm$ 0.54	***0.000

**Graph 1 : Effect of Extract on Erythropoietic Parameters**





Values are mean  $\pm$  S.D

N=10= number of animals.

\*\*p<0.001 = more significant

\*\*\*p<0.0001 = highly significant

IS = insignificant difference

Following t-test and ANOVA df (3, 39)

#### DISCUSSION:

Herbal medicines have been used since ancient civilizations for treatment of various ailments. Phytochemicals are synthesized by plants which are chemical compounds. [19] Phytochemicals have biological significance includes antioxidants which may reduce risk of cancers, Alziemers, aging and are beneficial in other diseases too.[20] The present study has been conducted to explore the erythropoietic effects of ethanolic extract of Piper chaba.

It has been revealed by literature survey that polysaccharide has positive effects on hematopoietic activity. Granulocyte-Monocyte colony stimulating factor, Interleukin 6 stimulation is basically responsible for hematopoietic activity.[21]

Another literature research showed that when pure carbohydrate was isolated from Aloevera extract showed enhanced hematological activity. It was found that this carbohydrate also affected cytokine MRNA levels in liver and lung which caused release of granulocyte colony stimulating factor (G-CSF) and stem cell factor (SCF) which are considered hematopoietic cytokines. [22]

The chemical constituents of Piper chaba extract show the presence of carbohydrates so it can be postulated that Piper chaba possesses hematopoietic effects.

Table 1 and Graph 1 shows the effect on erythropoietic activity of ethanolic extract of Piper chaba and control group after 7 day dosing.

The results showed increased hemoglobin levels after 7 day dosing of Piper chaba as compared to control. Hemoglobin is found in the RBC'S of humans. It is a metalloprotein containing Iron and plays a role in oxygen transport. Piperine to some extent maybe responsible for this effect i.e stimulation of erythropoietic system. [23]

The above results are also supported by the experimental data of RBC'S. The RBC'S count was also slightly elevated by Piper chaba. RBC'S are an important part of complete blood count and is utilized as an indicative tool to detect "Anemia" in which there is decrease in red cell count.[24]

Hematocrit also known as packed cell volume denotes the percentage of volume of whole blood that is made up of RBC'S. The number and size of RBC'S influence the hematocrit measurement. Our experimental study shows increase level of hematocrit by Piper chaba [25]. This further confirms our results that Piper chaba possesses positive effects on erythropoietic system.

Mean corpuscle volume is the average red blood cell volume on basis of size. Piper chaba extract showed decreased in MCV [26]. This result is indicative of the fact that the body's compensatory mechanism is working by stimulating erythropoietic system maybe due to deficient oxygen transport to cells.

Mean cell hemoglobin is defined as hemoglobin per red blood cell in blood cell [25]. Our results showed increased MCH values by the extract. This is related to fact that hemoglobin concentration more so MCH is increased more too.

Mean corpuscular hemoglobin concentration is defined as concentration of hemoglobin in given volume of red blood cells. Our results showed increase levels of MCHC in the extract which may be due to increase RBC'S count and increased hemoglobin. The presence of carbohydrates in this extract might be reason of stimulated erythropoietic activity [22].

#### **CONCLUSION:**

From the above study it can be concluded that ethanolic extract of fruit of Piper chaba contains polysaccharides and piperine which are responsible for erythropoietic activity of extract. This knowledge is beneficial as it can be used in treatment of anemia.

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