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EVALUATION OF ANTI ANAEMIC ACTIVITY OF AQUEOUS *PIPER BETLE* LEAF EXTRACT AGAINST PHENYL HYDRAZINE INDUCED ANAEMIA IN WISTAR RATS

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ABSTRACT

The aim of the present study was to investigate the anti-anaemic activity of aqueous leaf extract of Piper betle against Phenyl hydrazine induced haemolytic anaemia in Wistar rats. Hemolytic anaemia was induced by intra-peritoneal administration of phenyl hydrazine at a dose of 40 mg/kg for 2 consecutive days. Anaemic rats were orally treated with aqueous extract of Piper betel at doses of 250, 1000 & 2000 mg/kg/day. The rats were analyzed for hematological parameters such as Hb, RBC and hematocrit on day 0, 2 and 21.The aqueous extract significantly increased the RBC, Hb, Hematocrit levels which conclude that it exhibits anti-anaemic activity. This study revealed antianemic activity of aqueous extract of Piper betle, thus confirming the traditional use of this plant in anemia treatment.

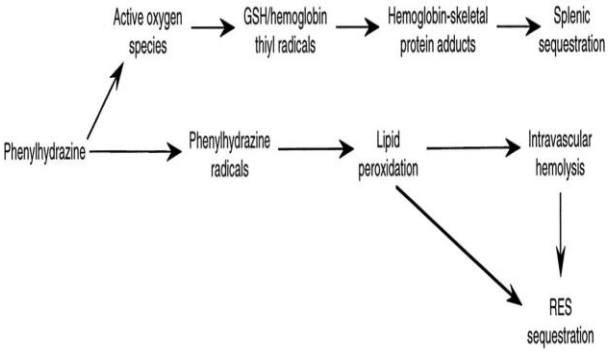
KEYWORDS: Anaemia, Hematological Parameters, Phenylhydrazine, Piper Betle, Hematological Parameters.

INTRODUCTION

According to WHO anaemia is a condition that develops when blood lacks enough healthy RBC or haemoglobin. It is characterized by a decrease in Haemoglobin Concentration (Hb), Red Blood Cells Count (RBC) and Packed Cell Volume (PCV).[1,2] It affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants. It is a condition, wherein the quantity of circulating haemoglobin (Hb) in the blood is <13 g/dl for male and <12 g/dl for female adult. Therefore, there is a need for proper management of micro nutrient deficiencies, especially iron.[3]

There are many types of anaemia[4], many of which are rare but in all cases there is lower than normal number of circulating red blood cells.

Hemolytic anaemia is a form of inherited or acquired anaemia resulting from either intravascular or extravascular RBC destruction. It has numerous external and internal causes which are either relatively harmless or are life-threatening in nature.[5] The exposure to many chemicals has also been associated with RBC destruction and haemolytic anemia.[6] The hemolytic activity of aryl hydrazines, such as phenyl hydrazine, dapsone, hydroxylamine, divicine, may lead to acute haemolytic anemia in vertebrates.[7]



Mechanism of Phenylhydrazine induced Anaemia.

Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometimes offer better therapeutic value than synthetic drugs.[8]

Piper betle (Family: Piperaceae) (Vedic name: saptasira) is one such widely growing tropical plant. Its leaves contain strong pungent and aromatic flavour. It is widely consumed as a post meal mouth freshener and is traditionally credited with wound healing and digestive and pancreatic lipase stimulant activities.[9]

It relieves head ache, scanty or obstructed urination as diuretic, weakness of nerves, sore throat, anti ulcer activity. It was evaluated the anti ulcer activity of hydro-alcoholic extract of piper betle (HEPB) in rats.[10] The

piper betle extract possesses a coagulant activity. The ethanol extract contained in the piper betle could stimulate clotting in the blood cells. It is caused by the increase of blood plasma concentration which further escalates the plasma fluid into the blood cells.[11]

MATERIALS AND METHODS

MATERIALS

Chemicals and Reagents: All chemicals and reagents used were of analytical grade. Pheylhydrazine hydrochloride (CAS Number: 59-88-1, Product Number: 201708003) was purchased from Chemist Laboratories, Hyderabad.

Plant Collection and Identification: The leaves of the plant were collected from local market, Hyderabad. The plant was authenticated as *Piper betle L*. belonging to the family Piperaceae by Dr.Madhava Chetty, Professor and Head, Department of Botany, Osmania University, Hyderabad with a (voucher number. 300). The leaves were washed and air dried at room temperature. Dry samples were ground and the powdered sample stored for further use.

Experimental Animals: A total of 36 wistar rats of either sex weighing between 150-200 g were obtained and kept in well aerated laboratory cages in the Biolab, Sanzyme Pvt Ltd, Hyderabad (CPCSEA NO. 1688/PO/Rc/S/13/ CPCSEA). The animals were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed with water and Feed *ad libitum.*

METHODS

Preparation of extracts: 100 g of plant powder was subjected to boiling in one Litre of distilled water for ten minutes. The decoction was filtered twice on absorbent white cotton then once on Whatman filter paper N°3. The filtrate was concentrated and dried to 40°C with oven and preserved at a temperature of –4 °C.[12]

Acute Oral Toxicity study (LD_{50})

The acute toxicity study was performed according to the OECD guideline 423 and the dose for the subsequent study was selected based on the LD_{50}.[13]

Anaemia induction: Anaemia was induced in rats by intraperitoneal administration of 40 mg / kg / day of phenylhydrazine (PHZ) for two days (D0 and D1).[14] The treated rats with phenylhydrazine whose haemoglobin concentration <13 g / dl were considered as anemic and included for the study.

Experimental design: The animals were randomly distributed according to weight in seven groups each of six rats and received *Piper betle* extract and Vit B_{12}(reference drug) by oral route as follows.

The extracts and Vit B_{12} treatment began two days after phenyl hydrazine administration.

- Group I (Normal): normal control received distilled water for 21 days.
- Group II (PHZ control): anemic control received phenylhydrazine (40 mg/kg) at days 0 (D0) and 1 (D1) then distilled water daily during 21 days.
- Group III (LPBE): rats treated with phenylhydrazine (40 mg/kg) at day 0 (D0) and day 1 (D1) then daily of *Piper betle leaf* aqueous extract (250 mg/kg) daily during 21 days.
- Group IV (MPBE): rats treated with phenylhydrazine (40 mg/kg) at day 0 (D0) and day 1 (D1) then daily *Piper betle leaf* aqueous extract(1000 mg/kg) daily during 21 days.
- Group V (HPBE): rats treated with phenylhydrazine (40 mg/kg) at days 0 (D0) and 1 (D1) then daily piper betle leaf aqueous extract (2000 mg/kg) daily during 21 days.
- Group VI (Vit B12): Standard control (reference) received phenylhydrazine (40 mg/kg) at days 0 (D0) and 1 (D1) and daily Vit B12 (1 mL/rat) during 21 days

Blood samples were collected on day 0, 2 and 21 from all the rats from the orbital plexus of eye in EDTA vials and evaluated for hematological parameters such as RBC, Hb, and packed cell volume (PCV).

Statistical analysis

The results were expressed as the Mean ± SEM. They were analysed using standard *t*- test and significance was observed at *P<0.05, **P<0.01 and ***P<0.001.

RESULTS AND DISCUSSION

Acute toxicity test: Studies revealed the plant extract to be safe up to the dose of 2000 mg/kg body weight.

Antianaemic Activity

Table 1: Effect of aqueous extract of *Piper betle* leaves on red blood cells (10^6 µg/ml)number in phenylhydrazine-induced anemia in rats.

	RBC(10^6 µg/ml)			
	Groups	**Day 0**	**Day 2**	**Day 21**
I	Normal control(Distilled water)	7.12±0.19	7.49±0.15***	7.25±0.27***
II	Positive control(PHZ40 mg/Kg)	7.62±0.19	3.66+0.19***	4.45±0.34***
III	LPBE(250 mg/kg) +PHZ(40 mg/kg)	7.70±0.13	3.98±0.25***	5.90±0.39***
IV	MPBE(1000 mg/kg)+ PHZ (40 mg/kg)	7.61±0.12	3.95±0.16***	6.61±0.39***
V	HPBE(2000 mg/kg)+ PHZ (40 mg/kg)	7.88±0.18	3.95±0.25***	6.90±0.41***
VI	Standard Control Vit B_{12} (1ml/rat)	7.56±0.11	3.76±0.12***	6.99±0.35***

Values were expressed as mean ± SEM, with n=6 in each group. ***P<0.001; **P<0.01 *P<0.05.

Table 2: Effect of aqueous extract of *Piper betle* leaves on Haemoglobin (mg/dl) in phenylhydrazine-induced anemia in rats.

Haemoglobin (mg/dl)				
Groups		**Day 0**	**Day 2**	**Day 21**
I	Normal control(Distilled water)	14.84±0.02	14.10±0.15***	14.40±0.14***
II	Positive control(PHZ40 mg/Kg)	14.77±0.29	7.79±0.24***	11.11±0.35*
III	LPBE(250 mg/kg) +PHZ(40 mg/kg)	14.81±0.15	7.83±0.15***	12.05±0.21***
IV	MPBE(1000 mg/kg)+ PHZ (40 mg/kg)	14.46±0.32	8.16±0.14***	12.56±0.31***
V	HPBE(2000 mg/kg)+ PHZ (40 mg/kg)	14.76±0.17	8.06±0.25***	13.69±0.15***
VI	Standard Control Vit B_{12} (1ml/rat)	14.84±0.24	7.80±0.36***	13.65±0.35***

Values were expressed as mean ± SEM, with n=6 in each group. ***P<0.001; **P<0.01, *P<0.05.

Table 3: Effect of aqueous extract of *Piper betle* leaves on Hematocrit/ PCV(%) in phenylhydrazine-induced anemia in rats.

PCV (%)				
Groups		**Day 0**	**Day 2**	**Day 21**
I	Normal control(Distilled water)	43.14±0.35	41.72±0.19	43.33±0.1
II	Positive control(PHZ40 mg/Kg)	42.98±0.67	23.29±0.23***	34.85±2.46**
III	LPBE(250 mg/kg) +PHZ(40 mg/kg)	44.27±0.21	24.18±0.36***	40.94±0.88**
IV	MPBE(1000 mg/kg)+ PHZ (40 mg/kg)	45.05±0.30	23.06±0.05***	42.54±0.76*
V	HPBE(2000 mg/kg)+ PHZ (40 mg/kg)	44.83±0.38	22.57±0.40***	43.47±0.41**
VI	Standard Control Vit B_{12} (1ml/rat)	44.26±0.31	22.8±0.32***	45.47±0.24***

Values were expressed as mean ± SEM, with n=6 in each group. ***P<0.001; **P<0.01, *P<0.05.

The intraperitoneal administration of phenyl hydrazine at dose (40 mg/kg) during two successive days involved a significant decrease (P<0.001) of RBC (10^6/µL) (51.77%), hemoglobin concentration (mg/dl) (47.35%) and haematocrit (%) (45.72%) in comparison to day 0 parameters.

The aqueous extract of Piper betle extract treatment for 21 days exhibited dose dependent and significant improvement in haematological parameters like RBC, Haemoglobin and Hematocrit (PCV) when compared to Group II (Positive control). The activity of high dose of test extract (2000 mg/kg) was comparable to that of Group VI (Reference control group, i.e., Vit B12).

The aqueous extract of *Piper betle* extract treatment for 21 days exhibited dose depeSndent and significant improvement in RBC count (10^6/µL) at three dose levels i.e., Group III (LPBE 250mg/kg) (51.11%, P<0.01), Group IV (MPBE 1000mg/kg) (74.78%, P<0.001), Group V (HPBE 2000mg/kg) (77.88%, P<0.001) when compared to Group II (PHZ Positive control). The effect of high dose of PBE (2000 mg/kg) was comparable to that of Group VI(Reference control group, i.e., Vit B12) (87.65% P<0.001).

The aqueous extract of *Piper betle* extract treatment for 21 days exhibited dose dependent and significant improvement in Haemoglobin count (mg/dl) at three dose levels i.e., Group III (LPBE 250mg/kg) (54.20% increase (P<0.05), Group IV (MPBE 1000mg/kg) (54.28% increase, P<0.001), Group V (HPBE 2000mg/kg) (70.36% increase, P<0.001) when compared to Group II (PHZ Positive control). The effect of high dose of PBE (2000 mg/kg) was comparable to that of Group VI (Reference control group, i.e., Vit B12) (76.95% P<0.001).

The aqueous extract of *Piper betle* extract treatment for 21 days exhibited dose dependent and significant improvement in Hematocrit (%) at three dose levels i.e., Group III (LPBE 250mg/kg) (90.11%, P<0.01), Group IV (MPBE 1000mg/kg) (90.18%, P<0.05), Group V (HPBE 2000mg/kg) (90.19%, P<0.01) when compared to Group II (PHZ Positive control). The effect of all the doses of PBE (250, 1000 & 2000 mg/kg) was comparable to that of Group VI (Reference control group, i.e., Vit B12) (89.61% P<0.001).

DISCUSSION

Phenylhydrazine is a non-immunogenic drug that induces changes in the RBC membrane, which leads to oxidative denaturation of Hemoglobin. The final effect of the denaturation is the formation of an altered Hb known as “Heinz bodies” which decreases the life span of the erythrocytes.[15] This is characterized by a significant increase in the incidence of micro-nucleated polychromated and hypochromic erythrocytes resulting in increased mean cell volume and decreased mean cell Hb concentration levels.[16,17] Altered erythrocytes are removed by the spleen and liver of the reticuloendothelial system resulting in a compensated hemolytic anemia. PHZ-induced anemia is one of the model for the study of hematinic effects of drugs.[18-21]

Treatment with different doses of Piper betle extract resulted in increased values of these parameters significantly. The Hb concentration was found to be

increased than the positive control animals at the end of the treatment. This indicates that the aqueous extract of Piper betle contains some bioactive molecules like alkaloids, cardiac glycosides, flavonoids, saponins, tannins, terpenoids, etc.[22] which prevent or repair the damage done to the cells by free radicals or highly reactive oxygen species.

A preliminary study has reported that Piper betle leaf extract contains large number of bioactive compounds. It was reported that fresh leaves contain moisture- 85.4%, protein- 3.1%, fat- 0.8%, carbohydrates- 6.1%, fibre- 2.3%, calories- 230mg, phosphate- 40mg, iron- 7mg, iodizable iron- 3.5mg, iodine- 3.5 microns, potassium nitrate- 0.26-0.42%, sugar (glucose, fructose, maltose, sucrose), enzymes (diastase, catalase.[23] It was reported to have Monoterpenes: Trans-sabinene hydrate (tr); Sesquiterpenes: E-caryophyllene(0.4%), δ-cadinene(tr), α-humulene(tr), γ-murolene(tr); Alcohols: α-cadinol (tr), τ-muurolol (tr); Esters: Methylsalicylate, chavebetolacetate(11.7%); Aldehyde: decanal(tr); Phenols: Chavicol(0.4%), eugenol(0.4%), chavebetol(80.5%).[24]

The anti-anemic property of Piper betle may be attributed to the presence of the above-mentioned bioactives as they are known to exert anti-oxidant activity as reported in the literature. From our study, it can be established that the anti-anemic potential of Piper betle can be explored further in developing a novel remedy for anaemia.

CONCLUSION

The collective data of this study revealed that *Piper betle* leaf aqueous extract has considerable anti-anemic activity as shown in PHZ-induced anemia in experimental rat model indicating the use of this plant for the treatment of anemia. Further studies are required to precisely define the bioactive and to develop suitable formulations to ensure maximum bioavailability and therapeutic efficacy.

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