J. Pharm. Res. 2018;17(3):8–16

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

ORIGINAL ARTICLE

Potential Benefits of Tephrosia purpurea in Ana emia and Thrombocytopenia

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ARTICLE INFO

Article history:
Received 10.05.2018
Accepted 25.09.2018
Published 12.10.2018

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https://doi.org/
10.18579/jopcr/v17.3.anuja

ABSTRACT

Objectives: Anaemia is a common nutritional disease characterized by a decrease in haemoglobin and red blood cell (RBC) levels. Thrombocytopenia is a condition in which the platelet count decreases from the normal level and is characterized by excessive bleeding from the mouth, nose, and gums. The methanolic extract of Tephrosia purpurea contains flavonoids that show antioxidant and anti-anaemic activities. In the present study, we investigated the effect of Tephrosia purpurea on phenylhydrazine-induced anaemia and a heparin-induced thrombocytopenic rat model.

Methods: The pulverized form of the whole plant was subjected to Soxhlet extraction, followed by phytochemical investigation and antioxidant activity using the DPPH method. Anaemia was induced by phenylhydrazine 60 mg/kg i.p for two days, and then treated with various doses of Tephrosia purpurea (200 400 g/kg p. o.) for 14 days. Parameters like RBC, Hgb, and MCH were measured. Thrombocytopenia was induced with heparin 2000 IU/kg for 10 days, followed by treatment with various doses of Tephrosia purpurea (200-400gm/kg p.o) for 14 days. Various parameters such as platelet count, bleeding time, and clotting time were observed.

Findings: Tephrosia purpurea has excellent anti-anaemia and anti-thrombocytopenic activities. There was an increase in RBCs, Hbs, and WBCs in comparison to the phenylhydrazine-induced group, which also showed a dose-dependent increase in haematological parameters. In addition, Tephrosia purpurea showed excellent anti-thrombocytopenic activity. It showed a marked increase in platelets and a decrease in bleeding time and clotting time in comparison to the heparin-induced thrombocytopenia group.

Novelty: Tephrosia purpurea has potential and can be considered the drug of choice for the management of anaemia and thrombocytopenia.

Keywords: Tephrosia purpurea; Antianaemic activity; Antithrombocytopenic

INTRODUCTION

Anaemia is a common blood disorder which affects people of all ages and poses a significant threat to global healthcare. Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population of nine out of ten anaemia sufferers living in developing countries[1]. Thrombocytopenia is a bleeding disorder characterized by a decreased platelet count. The formation of antibodies against a structural platelet antigen is an immune system-mediated condition. In India, the most common cause of thrombocytopenia is dengue fever, in which platelets decrease due to platelet sequestration, destruction, and bone marrow suppression[2,3].

Tephrosia purpurea is an herbaceous perennial and branched plant found in tropical regions. This plant possesses various pharmacological as well as medicinal properties. Tephrosia purpurea is commonly used in Ayurveda traditional medicine in India to treat ailments such as heart, liver, spleen, and asthma[4]. The use of Tephrosia purpurea extract improved the cardiac histopathological changes and heart rate in diabetic rats by reversing the disarray and degeneration of fibres and attenuating vacuole formation in the cardiac tissue, as demonstrated in STZ-induced diabetic rats compared to the untreated group[4]. T. purpurea displays a range of biologically active compounds, resulting in its antioxidant, antimicrobial, wound healing, antidiabetic, anticarcinogenic, anti-inflammatory, immunomodulatory,

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antiulcer, and diuretic properties. Therefore, the present study was conducted to evaluate the potential benefits of Tephrosia purpurea in the management of anaemia and thrombocytopenia in experimental rats.

METHODOLOGY

Collection and authentication of plant material

The whole Tephrosia purpurea was purchased from Mr. Chelladurai, a scientist in Botany Chennai, India. The material procured was in the pulverized form. The plant material was identified and authenticated by Dr. P.E. Rajasekharan, Principal Scientist, Division of Plant Genetic Resources**,** and the voucher herbarium specimen no: METP-313/KCP has been preserved in the Department of Pharmacognosy at Krupanidhi College of Pharmacy, Bengaluru, India.

Method of extraction of Tephrosia purpurea[5]

Pulverised plants (45 g) were placed in a Soxhlet extractor in a round-bottom flask. Methanol (80%) was used as the solvent for extraction. Methanol (400 mL) and distilled water (100 mL) were mixed and poured into a condenser to initiate the extraction process. The setup was placed in a heating mantle at 40–60˚C for 6 h. After the extraction, the solvent was recovered using the same process. Subsequently, the solvent was evaporated using a Rota evaporator. After extraction, the practical yield was calculated.

Preliminary phytochemical investigation[6,7]

The prepared extracts were subjected to phytochemical analysis and antioxidant capacity determination. The remaining extract was stored in a refrigerator until further use.

Test for carbohydrates

- **Fehling's test:** 1 mL of Fehling's A and Fehling's B solutions was mixed in a test tube and boiled for 1 min. The same volume of the test solution was added and heated in a boiling water bath for 5-10 min. The brick-red precipitate confirmed the presence of carbohydrates.
- **Benedict's test:** Equal volumes of Benedict's reagent and the test solution were mixed in a test tube and heated for 5 min in a boiling water bath. Yellow, green, or red solutions indicate the presence of reducing sugar.
- **Molisch's t est:** 2 mL of Molisch's solution with crude plant extract and 2 mL of concentrated H_2SO_4 was mixed and poured along the side of the test tube. The appearance of a violet ring at the inter-phase of the test tube indicates the presence of carbohydrates.

Test for proteins

- **Biuret test:** 3 mL of extract was added to 4% sodium hydroxide, and a few drops of 1% $CuSO_4$ solution were added. A pink or violet colour indicates the presence of a protein.
- **Millon's test:** 5 mL of Millon's reagent was added to 3 mL of extract, which led to white precipitates, indicating the presence of proteins.

Test for steroids and Terpenoids

- **Salkowski reaction:** 2 mL of chloroform and 2 mL of conc. H_2SO_4 was added to 2 mL of the extract and mixed thoroughly. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence which indicates the presence of steroids and terpenoids.
- **Libermann Burchard t est:** A few drops of acetic anhydride, were added to crude extract, then boiled and cooled. Concentrated sulphuric acid was then added to the sides of the test tube. Formation of a brown ring at the junction between the two layers was observed. The upper layer exhibiting a green tint and the lower layer displaying a deep red colour signify positive test results for steroids and triterpenoids, respectively.

Test for glycosides

- **Liebermann's test**: Acetic acid (2 mL) and chloroform (2 mL) were mixed with the crude plant extract. Subsequently, the mixture was cooled and combined with conc. H_2SO_4, resulting in the appearance of a green colour, which served as evidence for the presence of the aglycone steroidal component of the glycosides.
- **Salkowski's test**: 2 mL of con. H_2SO_4 was added to the crude extract of the entire plant. The reddish-brown colour indicates the steroidal aglycone part of the glycoside.
- **Legal's test:** 1 mL of pyridine was added to 1 mL of sodium nitroprusside, and the pink colour changed to red, indicating the presence of glycosides.
- **Keller Killiani test :** The test involved mixing the extract with a few drops of glacial acetic acid and ferric chloride solution, followed by the addition of con. sulphuric acid. The formation of two separate layers was observed: a lower reddish-brown layer and an upper acetic acid layer that turned bluish green. The presence of these layers indicated a positive test result for glycosides.

Test for flavonoids

- **Shinoda test:** To 3 mL of the extract, 5 mL of 95% ethanol and a few drops of conc. HCl and magnesium turnings (0.5 g) were then added. The pink colour indicated the presence of flavonoids.

- **Alkaline reagent t est**: The extract was treated with a sodium hydroxide solution and showed an increase in the intensity of the yellow colour which became colourless upon the addition of a few drops of dilute hydrochloric acid, indicating the presence of flavonoids.

Test for alkaloids

The aqueous alcoholic extract was evaporated, diluted with HCl, added to the residue, and properly mixed and filtered. The filtrate was tested for the presence of alkaloids.

- **Tephrosia purpurea Dragendroff's test:** A few drops of Dragendroff's reagent were added to 2-3 mL of filtrate, and orange-brown precipitates confirmed the presence of alkaloids.
- **Mayer's test:** A few drops of Mayer's reagent were added to 2-3mL of filtrate, the formation of the precipitates indicates the presence of alkaloids.
- **Hager's t est:** The extract was treated with a few drops of Hager's reagent (saturated picric acid solution). The formation of yellow precipitate shows a positive result for the presence of alkaloids.

Test for tannins

- **Ferric chloride t est:** A 5% $FeCl_3$ solution was added to 2-3 mL of the aqueous or alcoholic extract, and the formation of a deep blue-black colour indicated the presence of tannins.
- **Lead acetate solution t est:** The extract was treated with a few drops of lead acetate (10%) solution, resulting in the formation of a yellow precipitate, indicating the presence of tannins.

Test for saponins

- **Foam t est:** The stock extract solution (1 mL) was diluted with 20 mL of distilled water in a test tube and vigorously shaken. The presence of a foam layer on top of the test tube indicated the presence of saponins.

Test for vitamin c (Ascorbic acid

- **DNPH t est:** The extract was treated with dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate confirms the presence of vitamin C.
- 2 mL of water, 0.1 g of sodium bicarbonate, and 20 mg of ferrous sulphate were added to 2 mL of 2% w/v extract and allowed to stand, and a deep violet colour was formed. Subsequently, 5 mL of 1M H_2SO_4, was added and the violet colour disappeared, indicating the presence of vitamin C.

Antioxidant activity of Tephrosia purpurea determined by DPPH Radical scavenging activity[8]

The antioxidant activity of METP was measured using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. One millilitre of DPPH (0.01mM) was added to 3 mL of METP at various concentrations. The reaction mixture was then incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a blank. The free radical-scavenging activity of the plant extract was determined by comparison with that of the methanol control. A lower absorbance of the reaction mixture indicated a higher free radical scavenging activity.

Effect of METP on anaemia in experimental animal model

Female Sprague Dawley rats weighing 200± 50 g were housed at 25 ± 5°C in a well-ventilated animal house under a 12:12 h light/dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were maintained under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). All rats were provided a normal pellet diet and water ad libitum prior to dietary manipulation. The Institutional Ethical Committee approved the experimental protocol No.2016/PCOL/02/KCP/IAEC.

Induction of anaemia by phenylhydrazine (PHZ)[9]

A phenyl hydrazine hydrochloride solution was prepared in 0.1M potassium phosphate buffer (pH 7.4) and sterilized by filtration prior to use. Anaemia was induced by intraperitoneal administration of phenylhydrazine (60 mg/kg body weight) for 2 days.

Experimental groups for anemia[10,11]

- Group 1: Negative control, treated with vehicle 5 mg/kg p. o. for 14 days.
- Group 2: Positive control, treated with PHZ (60 mg/kg, i. p.) for 2 days.
- Group 3: Low-dose METP 200 mg/kg p. o. for 14 days after the induction of anaemia.
- Group 4: Medium dose of METP 300 mg/kg p. o. for 14 days after anaemia induction.
- Group 5: High dose of METP 400 mg/kg p. o. for 14 days after induction of anaemia.
- Group 6: Standard group; standard iron (Fefol capsule) 0.012 mg/kg p. o. for 14 days after induction of anaemia[12].

Estimation of RBCs[13,14]

Blood was collected from the rats into EDTA tubes/heparinized tubes using the tail vein method[13]. RBCs were estimated using Neubauer's chamber. The total

erythrocyte count was determined by hemocytometry following the method of Gottfried and Gerard[14–16].

Estimation of Hemoglobin by Sahli's hemoglobinometer (acid hematin method[17]

N/10 HCl was placed in a diluting tube to mark 20. Then, blood was pipetted up to the 20-cubic-mm-mark and blown into the diluting tube, and rinsed the pipette, after 10 minutes distilled water was added drop by drop until the colour matched exactly to the standard and noted the reading that indicated the haemoglobin percentage.

Mean Cell Haemoglobin[18]

Mean corpuscular haemoglobin (MCH) is the average amount of haemoglobin per red blood cell in a blood sample. MCH is used to diagnose the type, cause, and severity of anaemia. The normal range for MCH is 27-31 picograms/cell in adults.

Estimation of WBCs using Neubauer's Chamber[19]

WBCs counts were estimated using Neubauer's chamber.

Effect of METP on thrombocytopenia in an experimental animal model

Induction of thrombocytopenia by heparin[20]

A subcutaneous injection of low molecular weight heparin, at a dose of 2000 IU/kg, was injected into the rats daily for 10 days.

Experimental groups for Thrombocytopenia

- Group 1: Positive control (vehicle, 5 mg/kg) p. o. for 14 days.
- Group 2: Negative control Heparin 2000 IU/kg) s.c for 10 Days
- Group 3: Low dose of METP 200mg/kg p.o. For 14 days
- Group 4: Medium dose of METP 300mg/kg p.o for 14 days
- Group 5: High dose of METP 400mg/kg p.o for 14 days
- Group 6: Standard drug Prednisone 7mg/kg/day p.o for 10 days

Determination of platelet count by Haemocytometry[21,22]

Platelet count was determined by hemocytometry following the method described by Rees and Ecker (1923).

Clotting time[23]

Blood was drawn into capillary tubes. The time of appearance of the blood drop in the cut tail was recorded. The capillary glass tube was then placed between the palms of both hands for 30 s to maintain it at body temperature. After 30 s, the tube was removed and a small portion of the capillary tube was broken at regular intervals of 30 s until a thread of clotted blood appeared between the two pieces of the capillary glass tube. The time interval between the appearance of the drop of blood and the thread of the blood clot was considered as the clotting time of rats expressed in minutes.

Bleeding time[24]

Determination of bleeding time: The bleeding time was determined using the modified Duke's method. The animals were kept in restrainers, and the tail was exposed. The animal tail was cleaned using hot water, and the rectified spirit and its tip were punctured using a sterile needle and blotted on Whatman filter paper until the bleeding stopped. The bleeding time was recorded in seconds.

Statistical Analysis

All results are presented as mean $\pm$ SEM. The significance of the differences compared to the positive control groups was determined using one-way analysis of variance (ANOVA), followed by Dunnett's test using GraphPad Prism version 5. Statistical significance was set at $p < 0.05$.

RESULTS

Extraction

The pulverized form of the whole plant was subjected to extraction using a Soxhlet apparatus with 80% methanol, and the yield was 6.52%.

Preliminary Phytochemical Investigation

Extracts subjected to preliminary phytochemical investigation showed the presence of various phytoconstituents such as carbohydrates, flavonoids, alkaloids, tannins, and vitamin C. The results are presented in Table 1.

Antioxidant activity by DPPH Radical scavenging activity of Tephrosia purpurea

METP demonstrated a dose-dependent increase in free radical scavenging activity. The results are shown in Table 2 which illustrates a significant increase in the inhibition of DPPH radicals due to the scavenging activity of the extract. The free radical scavenging activity also increased with increasing extract concentrations in the range of 25-150μg/mL. The IC_{50} value of the extract was found to be 62.08. Whereas the IC_{50} value of ascorbic acid was found to be 15.27.

Assessment of Anti-anemic activity of Tephrosia purpurea

The anti-anaemic activity of METP against phenylhydrazine-induced anaemia in Sprague-Dawley rats was evaluated. Haemoglobin, red blood cell (RBCs, MCH, and western blot WBCs counts were measured. After treatment with METP for 14 days, the study observed an increase in RBC and Hb counts, body weight, and WBC,

Table 1: Preliminary

Sr. No.	Test	Observation	Result
1	**Test for alkaloids**		
a.	Mayer's test	Brown precipitate	++
b.	Dragendroff's test	Precipitate	++
c.	Hager's test	Yellow precipitate	++
2	**Test for carbohydrates**		
a.	Benedict's test	Green colour	+
b.	Molish test	Violet ring appeared	+
c.	Fehling's test	Brick red precipitate	+
3	**Test for glycoside**		
a.	Libermann's test	Green colour	+
b.	Salkowski's test	Reddish brown colour	+
4	**Test for steroid & terpenoids**		
a.	Salkowski reaction	Greenish yellow colour	++
b.	Libermannburchard test	Fluorescence green	++
5	**Test for saponins**		
a.	Foam test	No foam appeared	_
6	**Test for flavonoids**		
a.	Shinoda test	Pink colour	++
b.	Alkaline reagent test	Yellow colour	++
7	**Test for Protein**		
a.	Biuret test	No pink colour	-
b.	Millon's test	No red colour	-
8	**Test for tannins**		
a.	5%$FeCl_3$ solution	Deep blue colour	++
b.	Lead acetate	Yellow precipitate	++
9	**Test for vitamin c**	Yellow precipitate	++

'+' represents presence of compound, '++' shows more prominence in color formation and '-' represent absence of compound

Table 2: DPPH Radical scavenging activity of METP

METP			Ascorbic acid		
Concentration (μg/ml)	**Absorbance**	**% DPPH Inhibition**	**Concentration (μg/ml)**	**Absorbance**	**% DPPH Inhibition**
25	0.948	21.46	5	0.559	16
50	0.661	46.72	10	0.626	30
75	0.50	59.67	15	0.727	51
100	0.302	75.63	20	0.801	66
125	0.106	91.42	25	0.886	84
150	0.022	98.21	30	0.956	98.4

and a decrease in MCH when compared to the negative control group. At all three doses at a low dose, mid dose and high dose there was an increase in RBCs, Hb, and WBCs in a dose-dependent manner, demonstrating the effectiveness of the anti-anemic property of Tephrosia purpurea (Table 3).

Assessment of Anti-thrombocytopenic activity of Tephrosia purpurea

The anti-thrombocytopenic activity of METP in heparin-induced anaemia in Sprague Dawley rats was evaluated. The platelet count, bleeding time, and clotting time were recorded. After treatment with METP for 14 days at a low dose (200 mg/kg), mid-dose (300 mg/kg), and high dose (400 mg/kg), there was an increase in body weight and platelet count and a decrease in bleeding time and clotting

Table 3: Anti-anemic activity of METP

Sr. No.	Groups	Body Weights (g)	Hgb (gm%)	RBCX10^{12} /L	MCH (pg/cell)	WBCX10^{9} /L
1	Negative Control	221.7±11.08	13.10±0.1528	7.328±0.2524	17.98±0.6526	9.68±0.33
2	Positive control (PHZ 60mg/kg i.p 2 days)	186.7±3.333	8.433±0.3774	2.670±0.3757	36.23±7.232	5.01±90.18
3	Low dose of METP (200mg/kg p.o 14 days)	205.0±4.282	9.633±0.2092^{c}	4.855±0.6801^{c}	21.50±2.461^{c}	4.48±0.23
4	Medium dose of METP (300mg/kg p.o 14 days)	203.3±4.216	11.07±0.3127^{a}	5.992±0.6157^{a}	19.44±1.953^{b}	5.89±0.16
5	High dose of METP (400mg/kg p.o 14 days)	200.0±3.651	12.13±0.2512^{a}	7.330±0.3062^{a}	16.74±0.9331^{b}	8.69±0.323^{a}
6	Standard (Fefol capsule 0.012mg/kg p.o for 14)	203.3±3.333	12.53±0.3333^{a}	6.175±0.6663^{a}	21.42±2.195^{c}	8.36±0.16^{a}

All Values are given as mean ± SEM, (n=6); a, b and c statistically significant at $p<0.05$ when compared low dose, medium dose, higher dose and standard group vs. positive control.
Note: a, b and c represent ***, **, * respectively.
All the results were calculated using ANOVA followed by Dunnett's' test by graph pad prism version 5.

Table 4: Anti-thrombocytopenic activity of METP

Sr. No.	Groups	Weight (g)	Platelets (10^{3} /mm3)	Bleeding time (sec)	Clotting time (sec)
1	Negative Control	208.3±3.073	756.0±40.16	75.83 ±3.005	123.3± 4.944
2	Positive control (Heparin 2000 IU/kg s.c for 10 Days)	225.0±4.282	412.5±40.41	116.7± 4.944	175.0± 7.638
3	Low dose of METP (200mg/kg p.o 14 days)	238.3±6.009	503.5±33.75	101.7± 4.773	151.7± 7.491^{c}
4	Medium dose of METP (300mg/kg p.o 14 days	248.3±6.009^{c}	542.2±43.65^{c}	90.00± 3.651^{a}	135.0± 4.282^{a}
5	High dose of METP (400mg/kg p.o 14 days)	255.0±6.191^{b}	641.0±25.40^{ag}	70.00 ±3.651^{a}	131.7± 4.773^{a}
6	Standard ((prednisone 7mg/kg p.o 10 days)	255.0±6.708^{b}	670.3±14.22^{a}	69.17 ±3.745^{a}	130.0± 3.651^{a}

All Values are given as mean ± SEM, (n=6); a, b and c statistically significant at $p<0.05$ when compared low dose, medium dose, higher dose and standard group vs. positive control.
Note: a, b and c represent ***, **, * respectively.
All the results were calculated using ANOVA method followed by Dunnett's test by using graph pad prism version 5.

time compared to the negative control. There was a dose-dependent increase in body weight and platelet count, and a dose-dependent decrease in bleeding and clotting times. Tephrosia purpurea showed anti-thrombocytopenic activity against heparin-induced thrombocytopenia in Sprague Dawley rats (Table 4).

DISCUSSION

The present study aimed to investigate the potential benefits of Tephrosia purpurea on anaemia and thrombocytopenia in Sprague-Dawley rats, followed by a phenylhydrazine-induced anaemia and heparin-induced thrombocytopenia animal model. The study was initiated with the authentication of Tephrosia purpurea, which belongs to the family Fabaceae. The pulverized plants were subjected to Soxhlet extraction to facilitate the extraction process. The solvent that was used for the extraction was methanol. Based on a literature review, methanol is an ideal solvent for extracting flavonoid and polyphenol compounds, the main phytoconstituent of interest for the expected pharmacological activity in future work[25]. Plant material produced a yield of 6.52%. A previous literature review confirmed the presence of alkaloids, phenols, flavonoids, saponins, proteins, carbohydrates, and tannins in Tephrosia purpurea extract[25] . The phytochemical estimation of the extract revealed the presence of carbohydrates, flavonoids, alkaloids, tannins, and vitamin C. The number of phytochemicals in the extracts provided various pharmacological activities. Plants can be used to treat various diseases because they contain many phytochemical constituents, such as phenolic and flavonoid compounds, which exhibit a variety of pharmacological activities. These plants can be used in several ways to promote and maintain good health. The present study includes the presence of carbohydrates, in which restriction plays a significant role in the modulation of lipid metabolism by stimulating lipase enzymes[26] . Furthermore, carbohydrates improve non-alcoholic fatty liver disease (NAFLD) compared with low-fat diets related to liver diseases. Therefore, the presence of carbohydrates

may influence METP for liver protection. The reducing sugar in the current investigation indicates the pharmacodynamic properties of Sicyos edulis, where the pharmacodynamic properties refer to METP, affinity towards receptors, protein of interest, and genetic expression. The extract contained phenolic and flavonoid compounds, which are known to exhibit antioxidant properties and help reduce the formation of various reactive oxygen species. ROS are thought to be involved in the pathogenesis of different diseases, and in the case of liver disease, they play a vital role in its development[27]. The current investigation on Tephrosia purpurea reported the presence of tannins. An antioxidant assay was performed using DPPH. The IC_{50} of the extract was 62.08. Therefore, in the proposed research, the antioxidant activity of the methanolic extract of Tephrosia purpurea will build a bridge between the pharmacodynamics and pharmacokinetics of chayote in preclinical studies on experimental laboratory animals.

A preliminary study of anti-anaemic activity was carried out using Tephrosia purpurea extract, followed by an in vivo phenylhydrazine model. Phenylhydrazine was administered and subjected to develop anaemia in Sprague Dawley rats. The use of phenylhydrazine resulted in decreased Hb and HCT levels, which lasted for 8–12 days[28]. Moreover, phenylhydrazine causes pathological alterations in various tissues, including the heart, kidneys, liver, and spleen[29]. Therefore, to screen for anti-anaemia potential of Tephrosia purpurea, we evaluated the levels of RBCs, Hb, and HCT during the experimental period.

RBCs are cellular components of the blood. The primary role of Red Blood Cells (RBCs) transport oxygen to body tissues. However, in certain pathological conditions, such as anaemia, the function of RBCs may be altered, which can negatively impact normal functioning of the body. In the current investigation, administration of phenylhydrazine resulted in a decrease in the red blood cell count of the blood during the experimental phase, a finding that aligns with previous findings[28,30]. This decrease in the RBC count was improved by treatment with Tephrosia purpurea. Phenylhydrazine causes selective destruction of mature RBCs through oxidative stress[31]. Therefore, the beneficial effect of Tephrosia purpurea on the RBC count may be due to its ability to prevent phenylhydrazine-induced haemolysis.

Haemoglobin (Hb) is a critical factor in evaluating the effectiveness of anti-anaemic drugs, as a decrease in Hb levels can result in a reduction in the oxygen-carrying capacity of blood. In this study, the administration of phenylhydrazine led to a decrease in Hb content during the experimental period, which was consistent with previous findings[28,30]. Improvement in the decline in blood Hb content was achieved through treatment with Tephrosia purpurea. Phenylhydrazine has been reported to induce anaemia by oxidative denaturation of Hb initiated by free radicals. Therefore, the antioxidant potential of Tephrosia purpurea may be responsible for the observed effect of RK on improving the blood Hb content.

HCT, also known as packed cell volume, is the ratio of the volume of packed RBCs to the total blood volume. A decrease in HCT is a common indication for anaemia. In this study, a significant decrease in HCT levels was observed in anaemic control rats as a result of phenylhydrazine-induced haemolysis. This finding is consistent with those of earlier reports[28,32]. The decrease in HCT levels was counteracted by T. purpurea treatment. This may be because of the protective effect of Tephrosia purpurea against phenylhydrazine-induced haemolysis.

Phenylhydrazine is known to stimulate the generation of reactive oxygen species, which can cause oxidative damage to red blood cells (RBCs). In contrast, flavonoids possess potent antioxidant properties that enable them to prevent or repair damage to RBCs[33]. Phytochemical analysis of Tephrosia purpurea revealed the presence of saponins, steroids, flavonoids, tannins, and phenolic compounds. This suggests that the anti-anaemia activity observed in Tephrosia purpurea may be attributed to the presence of flavonoids or other active compounds.

Heparin-induced thrombocytopenia (HIT) is a common drug-induced autoimmune disorder characterized by arterial and venous thromboembolism. Antibodies generated for immune complexes consist of platelet factor 4 (PF4), heparin, and immunoglobulin G (IgG)[34]. Thrombus formation is enhanced by monocytes and endothelial cells activated by HIT immune complexes, which induce the expression of tissue factors and generate thrombin, reinforcing immune-mediated platelet activation and procoagulant pathways[35,36]. The primary anticoagulant effect of heparin is achieved by inactivating thrombin and activated factor X (factor Xa) through a mechanism that depends on antithrombin (AT). Heparin binds to AT via a high affinity pentasaccharide sequence which inhibits factor Xa. Heparin not only prevents the formation of fibrin by inhibiting thrombin but also inhibits thrombin-induced activation of platelets and factors V and VIII[37–39].

The binding of high-affinity heparin to antithrombin is accompanied by a conformational change; this conformational change follows an initial, weak binding of heparin to antithrombin and causes a tight interaction between the polysaccharide and inhibitor, which is a prerequisite for heparin anticoagulant activity. According to some reports, conformational changes can result in a more favourable environment for the activity of proteases. The binding of the reactive site of antithrombin, which prompts a swift interaction with heparin, also encompasses coagulation proteases. Recent research suggests that this binding is not as strong or specific to antithrombin as previously thought. However, for certain enzymes, such as thrombin, Factor IXa and Factor Xia, it appears that an interaction between heparin and the protease, in addition to the polysaccharide

and antithrombin, is necessary for the accelerated inhibition of these enzymes.

Heparin functions as a catalyst in the antithrombin-protease reaction, which accelerates the activity of enzymes, such as thrombin, Factor IXa, and Factor XIa. It facilitates the interaction between heparin and non-stoichiometric amounts of these enzymes without being consumed during the reaction[40]. HIT is caused by antibodies that recognize a complex composed of heparin and platelet factor 4 (PF4) tetramers[41]. A platelet-specific chemokine is released in large amounts by activated platelets that bind heparin with high affinity and exists as a tetramer at concentrations found at the sites of platelet activation[42,43]. In the present study, the results showed an increase in the platelet count and a decrease in the bleeding time and clotting time when compared to the negative control, in which a dose-dependent increase in platelet count is seen[34,44,45].

Tephrosia purpurea is used to treat rheumatism, an immune-mediated disease; therefore, it was assumed that METP which decreases the antibody produced for the heparin-PT4 complex, destroys platelets[37]. β sitisterol, ursolic acid, spinosterol, epoxyflavon, and pongamol, may be responsible for its activity[38]. Another mechanism is by inhibiting the activity of antithrombin, stopping the inhibition of clotting factors X and IXa, or by increasing the platelet count[39]. METP can also be used as a preventive measure for dengue fever, which is characterized by a rapid decrease in platelets. METP possesses anti-viral activity and increases platelet counts due to its immunomodulatory action.

CONCLUSION

Tephrosia purpurea improved phenylhydrazine-induced decreases in RBCs, Hb, and HCT levels. Hence, Tephrosia purpurea has significant anti-anaemic activity against phenylhydrazine-induced anaemia and anti-thrombocytopenic activity against heparin-induced thrombocytopenia in rats. Further investigation into the quantitative determination of phytoconstituents, coupled with in vivo evaluations, is necessary to shed light on the precise mechanism underlying the observed anti-anemic and anti-thrombocytopenic effects.

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