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Original Article

Evaluating the Therapeutic Potential of Yemeni Shilajit from Dhamar, Ma'rib, and Raymah on Hemolytic Anemia in Rabbits: Effects on G6PD Activity, PCV, Hemoglobin, and RBC Count

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ABSTRACT

Background: Shilajit is a historically valued natural substance with promising but still preliminary scientific support for urinary and diabetic health, yet its growing popularity risks exploitation and exaggerated claims without rigorous human clinical validation. This study investigates the therapeutic effects of Yemeni Shilajit sourced from Dhamar, Ma'rib, and Raymah on hemolytic anemia experimentally induced in rabbits using phenylhydrazine.

Methods: Twelve male rabbits were divided into four groups: a control group and three treatment groups, each receiving Shilajit from one of the three regions. Anemia was induced via subcutaneous injection of phenylhydrazine hydrochloride, and treatment was administered orally at a dose of 125 to 250 mg/kg twice daily. Hematological parameters—including packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell (RBC) count, and glucose-6-phosphate dehydrogenase (G-6-PD) activity—were evaluated at various intervals.

Results: showed that Shilajit from Dhamar and Raymah significantly increased PCV, Hb, RBC count, and G-6-PD enzyme activity ($p < 0.05$), suggesting notable anti-anemic effects and potential for restoring hematological parameters to near-normal levels. In contrast, Ma'rib Shilajit exhibited limited efficacy, with significant improvement only in hemoglobin levels. Phytochemical analysis confirmed the presence of essential amino acids and bioactive compounds, such as fulvic acid and dibenzo-α-pyrones, which likely contribute to Shilajit's therapeutic action.

Conclusions: The study concludes that purified Shilajit from Dhamar and Raymah holds promise as a natural remedy for hemolytic anemia, supporting its traditional use and warranting further investigation for pharmacological applications.

Key words: Shilajit, phenylhydrazine, hemolytic anemia, Dhamar and Raymah

INTRODUCTION

Anemia is a common blood disorder affecting individuals of all ages, with increased risk among the elderly, young women of childbearing age, and infants. [1] Although anemia itself is not a disease, it frequently develops secondary to a variety of underlying conditions. There are over 400 types of anemia, many of which are rare, but all share the hallmark feature of a lower-than-normal number of circulating red blood cells (RBCs) or decreased hemoglobin levels. [2]

Typically, anemia is defined by reductions in hemoglobin concentration (Hb), RBC count, or packed cell volume (PCV) below established normal values. [3] The World Health Organization defines anemia in populations living at sea level as hemoglobin levels below 13 g/dL in men and below 12 g/dL in women. [4] Among the various forms of anemia, this study focuses on hemolytic anemia, which results from the abnormal breakdown of RBCs (hemolysis) either within blood vessels (intravascular) or in other parts of the body (extravascular). Hemolytic anemia can arise from numerous causes, ranging from benign to life-threatening conditions. [5]

Access to effective pharmaceutical treatments for anemia is often limited in rural populations worldwide, leading many to rely heavily on traditional herbal medicines for management. [6] Given the high prevalence of anemia and its projected increase, there is a critical need to explore more accessible, cost-effective, and safe treatment options. Traditional medicine practitioners have long claimed success in treating anemia with various plant materials. [7] For instance, in China, plant-based remedies have been used historically to treat blood disorders, including anemia, circulatory malformations, and hemorrhages. [8]

One such traditional remedy is Shilajit, a natural herbomineral substance widely used in Ayurveda and other traditional medicine systems. [9] Shilajit has demonstrated hematinic (blood-enhancing) activity and has been reported to be effective in treating sickle cell anemia and other blood disorders. [10] It is believed to contribute significantly to health improvement and disease prevention, yet scientific evidence validating its anti-anemic potential remains limited. Motivated by these traditional claims and preliminary reports, this study aims to investigate the anti-anemic effects of purified Shilajit sourced from Yemen.

MATERIALS AND METHODS

Sample collection and purification

Four types of Yemeni Shilajit samples, from Dhamar, Ma'rib, and Raymah, were collected. Approximately 3 kg of each sample, in the form of large hard rocks, were gathered and then ground into fine powder using a metallic mortar and pestle.

Purification was performed based on Shilajit's solubility in distilled water at a ratio of 1 g per 10 mL. A 30% solution of each sample was prepared, as this concentration is known to provide long-term stability, with an almost indefinite shelf life (Anastasia Artemva, 2001). The purification process involved dividing each powdered sample into precise 20-g portions, which were then placed into 200-mL conical flasks. These flasks were shaken on a rotary shaker at 150 rpm for 30 minutes to ensure complete dissolution. The solutions obtained were filtered to remove insoluble impurities, initially through loose cotton and subsequently through Whatman filter paper No.1. Following filtration, 100 mL of the purified solution was subjected to freeze-drying to obtain dry Shilajit. The resulting solid was collected and ground into a very fine powder using an electric grinder, then prepared in a suitable form for administration to rabbits.

Experimental animals

Twelve healthy male rabbits, aged from weaners to adults, were obtained from the University of Sana'a animal house unit. They were housed in the experimental animal room and fed exclusively on pelleted alfalfa and clean drinking water. The rabbits were acclimatized for one week before the experiment.

They were randomly divided into four groups of three rabbits each:

- Group A (Control): Received only distilled water.
- Group B: Treated with Dhamar Shilajit.
- Group C: Treated with Raymah Shilajit.
- Group D: Treated with Ma'rib Shilajit.

Shilajit was administered orally as a solution in water at doses of approximately 125 to 250 mg/kg, twice daily, to treat experimentally induced anemia.

Anemia induction

Anemia was induced following the method of Harris and Kugler (1971), with modifications by Sanni et al. (2005). Briefly, all rabbits received subcutaneous injections of 2.5% neutralized phenylhydrazine hydrochloride (Fisher Scientific, New Jersey) at an initial dose of 30 mg/kg body weight. Maintenance doses of 10, 15, and 20 mg/kg were administered every 3 days throughout the experiment.

Blood collection

Blood samples were collected from the prominent ear vein using sterile syringes, with 1 mL drawn into tubes containing EDTA for hematological analysis and 4 mL collected into plain tubes without anticoagulant for serum separation. The 1 mL EDTA samples were thoroughly mixed to prevent coagulation and used for hematology tests. The 4 mL samples were allowed to clot at room temperature, then centrifuged at 1000 rpm for 10 minutes to separate the serum, which was subsequently stored at −20°C until needed for biochemical assays.1 mL of blood was collected before anemia induction, after induction, and during treatment; 4 mL of blood was collected at the end of treatment.

Hematological examination

PCV, Hb, and RBC count were determined from whole blood samples according to Stoskopf's method.

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency screening was performed using a semi-quantitative fluorescent spot method (Randox Laboratories Ltd, UK). Deficient samples underwent further quantitative spectrophotometric analysis using a G-6-PD assay kit (Randox Laboratories Ltd). Additionally, hematological parameters (PCV, Hb, RBC) were measured in 15 blood samples from experimental rabbits across all groups using a hematology analyzer (Mythic 18, Orphée Company, Switzerland) at the National Central Public Health Laboratory (NCPHL) to assess hemolytic anemia.

Amino acid analysis

Amino acid composition of Shilajit was analyzed at the National Research Center, Egypt, using High-Performance Liquid Chromatography (HPLC) and an amino acid analyzer (LC300, Eppendorf, Germany).

Statistical analysis

Data were analyzed using SPSS version 17.0. Results are expressed as mean ± SD for normally distributed variables. Differences between groups were tested using an independent samples *t*-test, with significance set at $p < 0.05$.

RESULTS

Phytochemical screening of the purified Shilajit samples (**Table 1** and **Figure 1**) revealed the presence of valuable constituents, particularly peptides composed of both protein and non-protein amino acids such as aspartic acid, glutamic acid, glycine, valine, serine, tyrosine, threonine, histidine, lysine, and leucine. These amino acids include both essential and non-essential types and play a vital role in physiological functions. The hematological assessment following induction of anemia through phenyl hydrazine hydrochloride revealed a reduction in PCV by more than 50% from baseline in all rabbits by the second day, confirming the successful induction of anemia. However, treatment with the purified Shilajit of Raymah and Dhamar samples resulted in a significant ($p < 0.05$) increase in PCV values compared to the control group (**Table 2**). Furthermore, **Table 3** indicates that daily oral administration of these two samples significantly elevated RBC counts in anemic rabbits, and **Table 4** demonstrates that all three purified Shilajits (Raymah, Dhamar, and Ma'rib)

Table 1: Phytochemical components of Shilajit.

Type of amino acid	Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Valine	Leucine	Tyrosine	Histidine	Lysine
Cone. (ug/mL)	31.91	7.90	8.40	148.57	170.87	13.34	3.85	14.86	-	4.16

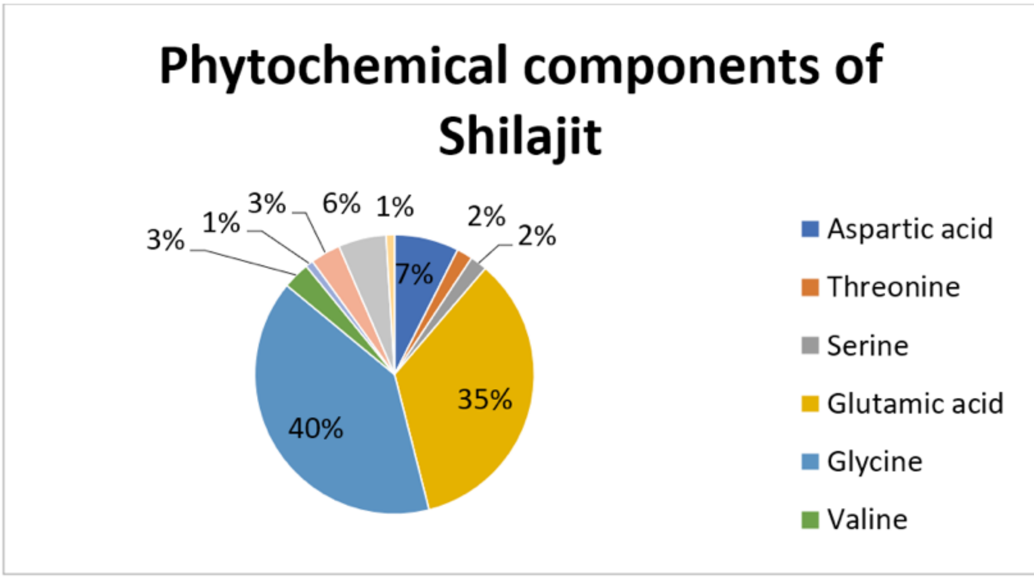

Figure 1: Phytochemical components of Shilajit.

Table 2: Effect of purified Shilajit on packed cell volume (%) of phenyl hydrazine-induced anemia in rabbits.

Duration of experiment (days)	Groups of rabbits			
	(A) Treatment with distilled water	(B) Treatment with Dhamar Shilajit	(C) Treatment with Raymah Shilajit	(D) Treatment with Ma'rib Shilajit
0	29.0 ± 3.0	31.7 ± 0.5	29.7 ± 0.59	29.0 ± 2.0
2	10.0 ± 1.0	13.7 ± 4.	12.0 ± 2.0	13.7 ± 0.59
6	19.3 ± 2.1	25.7 ± 2.5	19.7 ± 1.5	22.7 ± 5-5
9	26.0 ± 1.0b	23.3 ± 1.2	31.0 ± 1.0	20.3 ± 1.5
12	26.0 ± 1.0	21.7 ± 0.59	26.7 ± 0.6	22.02 ± 0
16	17.7 ± 0.59	20.0 ± 2.0	19.7 ± 0.59	17.0 ± 2.0

Values are expressed as mean ± SD ($n = 3$). Means within the same row sharing the same superscript are significantly different at $p < 0.05$.

Table 3: Effect of purified Shilajit on red cell counts (x1012/L) of phenyl hydrazine-induced anemia in rabbits.

Duration of experiment (days)	Groups of rabbits			
	(A) Treatment with distilled water	(B) Treatment with Dhamar Shilajit	(C) Treatment with Raymah Shilajit	(D) Treatment with Ma'rib Shilajit
0	3.8 ± 0.25	4.3 ± 0.52	3.4 ± 0.56	3.5 ± 0.67
2	2.9 ± 0.24	2.9 ± 0.36	2.6 ± 0.27	2.7 ± 0.49
6	1.8 ± 0.27	1.9 ± 0.45	2.1 ± 0.28	1.9 ± 0.61
9	2.7 ± 0.1	3.0 ± 0.25	3.4 ± 0.14	2.6 ± 0.10
12	3.2 ± 0.32	3.2 ± 0.40	3.6 ± 0.10	3.10 ± 0.51
16	2.2 ± 0.3	2.3 ± 0.29	3.1 ± 0.1	2.2 ± 0.42

Values are presented as mean ± SD (n = 3). Means in the same row bearing the same superscript are significantly different at $p < 0.05$.

Table 4: Effect of purified Shilajit on hemoglobin concentration (g/dL) of phenyl hydrazine-induced anemia in rabbits.

Duration of experiment (days)	Groups of rabbits			
	(A) Treatment with distilled water	(B) Treatment with Dhamar Shilajit	(C) Treatment with Raymah Shilajit	(D) Treatment with Ma'rib Shilajit
0	12.58 ± 0.8	13.15 ± 1.12	12.41 ± 0.99	12.71 ± 0.88
2	16.61 ± 3.74	17.58 ± 2.90	17.86 ± 1.87	17.38 ± 0.77
6	12.5 ± 2.29	11.2 ± 1.65	11.2 ± 1.39	9.1 ± 2.35
9	12.43 ± 1.18	14.74 ± 0.6	14.21 ± 0.17	14.14 ± 0.1
12	15.68 ± 0.44	17.67 ± 1.57	17.38 ± 0.17	17.05 ± 0.1
16	11.96 ± 1.85	14.45 ± 1.8	14.15 ± 1.05	13.35 ± 0.28

Values are expressed as mean ± SD (n = 3). Values in the same row sharing the same superscript are significantly different at $p < 0.05$.

Table 5: Effect of purified Shilajit on G-6-PD IU/g RBC, activity G-6-PD IU/g (RBC).

Duration of experiment (days)	Groups of rabbits			
	(A) Treatment with distilled water	(B) Treatment with Dhamar Shilajit	(C) Treatment with Raymah Shilajit	(D) Treatment with Ma'rib Shilajit
0	9.6	9.1	8.9	9.2
2	1.3	1.2	1.1	1.3
6	2.1	3.1	2.3	2.2
9	4.4	5.7	5	4.6
12	7.8	8.9	8.1	8
16	9.2	10.8	9.8	9.1

significantly ($p < 0.05$) increased hemoglobin concentrations compared to the control. Additionally, the study showed that treatment with the three Shilajits led to a significant rise in G-6-PD levels in treated rabbits compared to the control (**Table 5**), indicating a potential protective effect against oxidative damage.

DISCUSSION

Anemia was confirmed by a greater than 50% reduction in PCV values two days after phenyl hydrazine administration, aligning with earlier studies, such as that by Bowman and Rand (1980), which demonstrated chemically induced anemia in rats. Interestingly, by day 9, control animals showed signs of recovery, likely due to the diminishing effects of phenyl hydrazine before a booster dose. The chemical is known to induce Heinz body formation in RBCs, offering temporary protection from further hemolytic damage. In contrast, hematological parameters were significantly improved in the groups treated with purified Shilajit from Raymah and Dhamar, indicating their strong anti-anemic potential. The Ma'rib sample, although it showed some efficacy, only significantly increased hemoglobin concentration without a marked effect on other hematological indices.

These differences may be attributed to the phytochemical composition of each sample. Purified Shilajits from Raymah and Dhamar likely contain higher concentrations of bioactive compounds such as oxygenated dibenzo-α-pyrones, humins, humic acids, tirucallane triterpenes, phenolic lipids, tannoid-related metabolites, and fulvic acids, along with the previously mentioned amino acids and trace minerals. These compounds are known for their antioxidative and hematinic properties. Phenyl hydrazine induces oxidative damage via reactive oxygen species, but components such as fulvic acids and dibenzo-α-pyrones in Shilajit may counteract this effect, promoting RBC regeneration and stability.

Moreover, the significant increase in G-6-PD levels observed in all treated groups suggests that Shilajit enhances the antioxidant defense system of red cells, helping prevent oxidative hemolysis. This enzyme plays a key role in maintaining cellular redox balance, and its upregulation could be a crucial mechanism by which Shilajit mitigates anemia. The consistent hematological improvements without signs of protein metabolism disruption or nutritional deficiency suggest that the animals remained healthy throughout the treatment.

In conclusion, the findings support the traditional use of purified Shilajit, particularly from Raymah and Dhamar, in folk medicine for the management of anemia. These samples demonstrated potent anti-anemic effects through the restoration of PCV, RBC, hemoglobin, and G-6-PD levels, underscoring their therapeutic potential.

CONCLUSIONS

In conclusion, the findings of this study demonstrate that among the Shilajit samples tested, those from Dhamar and Raymah exhibit strong anti-anemic properties and are effective in the management of hemolytic anemia, while the Ma'rib sample showed only limited effects. The study confirmed that the bioavailability of minerals in Shilajit is enhanced by the presence of humic and fulvic acids, which aid in absorption and physiological activity. Amino acid analysis revealed that Yemeni Shilajit contains both essential and non-essential amino acids—including aspartic acid, glutamic acid, glycine, valine, serine, tyrosine, threonine, histidine, lysine, and leucine—along with small peptides that play a significant role in the repair and regeneration of blood cells.

Data from this study indicate that Yemeni Shilajit has potent anti-anemic effects by significantly increasing PCV and enhancing G-6-PD activity, which is particularly important in managing hemolytic anemia. The results further emphasize the metabolic and physiological importance of the amino acids present in Shilajit, underscoring its value as a natural health supplement.

Given its wide-ranging benefits, Shilajit is a versatile natural compound that holds promise in various pharmacological fields. It should be further investigated for its potential as an immunomodulator, aphrodisiac, or reproductive health support, and for its anti-allergic properties. Additionally, there is a strong potential for the development of pharmaceutical formulations of Shilajit for topical applications, such as in the treatment of burns and bone injuries. Future studies should also focus on quantifying the concentrations of humic and fulvic acids in Yemeni Shilajit to support its standardized use in pharmaceutical manufacturing.

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AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

None.

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