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The protective Effect of *Spinacia oleracea* L aqueous extract in mitigating anemia and general debility in experimental animals

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ABSTRACT

In this study, the effects of an aqueous extract of *Spinacia oleracea* L (SOAE) on experimentally induced anemia and its impact on muscle function have been investigated. Phytochemical analysis and HPTLC fingerprinting confirmed the presence of phenolic compounds and flavonoids in the extract. Oral administration of *S. oleracea* aqueous extract at a dose of 400 mg/kg extensively improved the erythrocyte count, hemoglobin, and hematocrit in the phenyl hydrazine-induced anemia model. We also evaluated the effect of adenine-induced chronic kidney disease (CKD) on hematological parameters along with the determination of serum erythropoietin and transferrin using an adenine-induced ELISA kit in rats. The adenine-induced CKD model showed a decrease in red blood cell count, hemoglobin level, hematocrit percentage, serum erythropoietin, and iron content while increase in transferrin level in the positive control group. Concurrent administration of adenine along with SOAE at doses of 200 mg/kg and 400 mg/kg effectively mitigated the extreme impact of *S. oleracea* aqueous extract on blood parameters. Co-administration showed significant improvement in the erythrogram, hemogram, hematocrit, erythropoietin, iron content, and transferrin levels in a dose-dependent manner. Moreover, it exhibited a positive impact on muscle coordination, demonstrating an increase in the time spent on the rotating rod and better grip strength compared to the control group. These findings suggest that *S. oleracea* aqueous extract could offer a natural remedy for addressing both anemia and general debility.

KEYWORDS: *Spinacia oleracea*, hematological parameters, erythropoietin, transferrin.

INTRODUCTION

Anemia is the most prevalent illness in women and girls, as well as in persons such as soldiers, athletes, and sports persons engaged in a profession, demanding strenuous physical work. Furthermore, anemia frequently results in a number of detrimental health conditions, including dyspnea, low immunity, and psychological issues including depression and diminished cognitive function (Myhre et al., 2016). In anemia, the oxygen-carrying capacity of the blood diminishes, which results in weakness, hampers physical tasks, work efficiency, fatigue, poor concentration, and reduced work productivity (Gledhill et al., 1985; Bassett et al., 2000; Dubnov et al., 2006). Globally, anemia is highly prevalent affecting 1.6 billion people, highest amongst children (40 %), during pregnancy (37 %), and women 15-49 years of age (30 %) (Tsai KZ *et al* 2019; WHO 2019; Li, Q et al 2019**).** It was estimated that one third of global population is suffering from anemia. It leads to weakness, fatigue, impaired work productivity, or difficulty concentrating (Gledhill et al., 1985). Furthermore, exercise capacity has been reduced during anemia because of impaired physical performance and decreased oxygen-carrying capacity of the blood (Bassett et al., 1985; Dubnov et al., 2006). The kidney is an intricate organ that performs a number of essential tasks. As stated by Bonventre et al. (2010), one of the roles of kidney is hormone synthesis, which is critical for preserving hematological balance. Red blood cell formation depends on the hormone erythropoietin. Anemia develops as a result of an erythropoietin deficiencies caused by renal insufficiency (Fisher, 1980). *Spinacia oleracea* (Family: Amaranthaceae), commonly known as spinach or Buai Leng, is traditionally recommended for the treatment of anemic, bacterial infections, convulsant, diabetic, helminthiasis, hyperlipidemia, inflammation (Choudhary et al., 2018), ulcer, and viral infections. It also has prophylactic use in nervous, hepatic (Jain et al., 2012), and respiratory diseases. Phytoconstituents found in *S. oleracea* are phenolic compounds (ortho-coumaric acid, para-coumaric acid, and ferulic acid), flavonoids (apigenin, glucuronide, jaceidin, flavone, kaempferol, myricetin, methoxyflavone, patuletin, quercetin, and spinacetin), carotenoids (lutein, β-carotene, violaxanthin, and neoxanthin), vitamins, and minerals (Jiraungkoorskul et al., 2016).

The aim of this study is to evaluate the effect of *S. oleracea* aqueous extract on anemia and general debility in experimental animals.

MATERIALS AND METHODS

Drugs and chemicals

2-2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (Sisco research lab, Mumbai, India), methanol (Research lab, Mumbai), phenyl hydrazine (Sigma Aldrich, Mumbai), Orofer XT (Emcure), adenine (Research lab fine chem. Industries, Islampur), ether (Sigma-Aldrich), Rat EPO ELISA kit, Rat transferrin ELISA assay kit (Krishgen Biosystems), Oxymetholone (Anadrol 50, Meditech). All chemicals and reagents used throughout the study were of analytical grade.

Plant material and authentication

Fresh leaves of *S. oleracea* were collected from the farm of Yadrav, Ichalkaranji, Kolhapur District, Maharashtra, India. A plant specimen was submitted for authentication, and it was

verified by botanist Dr. Vikas B. Awale from Bharati Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya, Sangliwadi, Sangli, Maharashtra, and Shivaji University, Kolhapur, Maharashtra (VSK 01).

Extract preparation

S. oleracea fresh leaves were washed and dried at room temperature for a period of two weeks. The 500 g of dried leaves were powdered and macerated with 5000 mL of water. *S. oleracea* aqueous extract (SOAE) was stored in the refrigerator for further experimental use. The percentage yield of the SOAE was calculated.

Phytochemical screening

Preliminary phytochemical screening of SOAE was performed to detect the presence of alkaloids, glycosides, tannins, saponins, triterpenoids, flavonoids, and phenolics (Trease & Evans., 1989).

In-vitro measurement of an antioxidant property

DPPH (Diphenyl-1-picrylhydrazyl and 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

In-vitro antioxidant activity of SOAE was measured by Diphenyl-1-picrylhydrazyl and 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (DPPH) radical scavenging activity. 100 µL of SOAE (1 mg/mL) was taken in the microtiter plate. To it, 100 µL of 0.1% methanolic DPPH was added and incubated for 30 minutes in a dark condition. Quercetin was used as a standard antioxidant. The mixture was then monitored for changes in color from purple to yellow, indicating a strong positive reaction, and from purple to pale pink, indicating a weak positive reaction. Plates were then analyzed using an Elisa plate reader at a wavelength of 490 nm (Vijayaraghavan et al., 2013; Prieto, 2012). Radical scavenging activity was calculated by the following equation: DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] X100

HPTLC fingerprinting

HPTLC finger printing studies were carried out according to the method of Wagner, Baldt, and Harbone. About 100 mg of an aqueous extract of SOAE was dissolved in 1 mL of distilled water, and it was centrifuged at 3000 rpm for 5 min. This solution was used as a test solution for HPTLC analysis. A Camag HPTLC system, comprising a Linomat 5 automatic applicator with a 100 µL syringe, a twin trough plate development chamber, Camag TLC scanner 3, and Server DESKTOP-5IHGUM1, version 3.1.21109.3 software, was used. The SOAE sample of about 7 µL was spotted in the form of bands having a bandwidth of 5 mm on a precoated silica gel 60 F254 HPTLC plate (10 × 10 cm, 250 µm thickness) (E. Merck, Mumbai, India). Spots were located 8mm from the bottom and 15 mm from the side edges and were allowed to dry for 5 minutes. Densitometric scanning was performed with a TLC scanner equipped with DESKTOP-5IHGUM1, version 3.1.21109.3 software (Camag) in reflectance absorbance. The slit dimensions were 6 mm × 0.45 mm, the scanning speed was 20 mms−1, and the data resolution was 100 µm/step. The mobile phase optimized and used was Ethyl Acetate: Water: Ethanol: Formic Acid (2: 7: 1: 3: 0.5 v/v/ v/v/v). Plates were scanned at 254 nm, which was selected

experimentally based on the distinctive absorption spectra of the compounds between 200 and 400 nm. Each plate was kept in the photo-documentation chamber (CAMAG) and captured the images at visible light and UV 366 nm and 254 nm. The peak numbers, with their height and area, peak display, and peak densitogram were identified. The retention factor (Rf) values at fingerprint data were recorded by DESKTOP-5IHGUM1, version 3.1.21109.3 software (Srinivasan et al., 2015).

Pharmacological evaluation and approval of research protocol

All the experimental protocols were reviewed by the Institutional Animal Ethical Committee (IAEC) of Biocyte Research & Development Pvt. Ltd. Ltd. Sangli, Maharashtra, India, was constituted as per the guidelines of the CPCSEA (Committee for Purposes of Control and Supervision of Experimental Animals), India (CPCSEA Guidelines). (OECD 420, 2001; Berger, 2007) (BiRD/Sangli/IAEC/23)

Experimental Animals

Female Wistar rats weighing between 200 gm ± 20% and aged between 8-12 weeks were chosen for the experimental study. They were housed in spacious cages provided with a standard diet and continuous access to food and water throughout the study. These cages were placed in a laboratory maintained at a temperature of 24 °C ± 1 °C, relative humidity of 45–55%, and a 12 h light–12 h dark cycle. Food was withheld for 3 hours before the commencement of the experiment.

Acute toxicity study

The acute oral toxicity study of the SOAE extract was conducted as per OECD 420 guidelines with a maximum single dose of 2000 mg/kg. Six female Wistar rats with a weight ranging between 200 gm ± 20% and an age between 8-12 weeks were selected for the study. The maximum single dose was administered by oral gastric intubation. After administration of the dose, each animal was observed for 30 minutes, intermittently for the next 24 h, and subsequently, every day for a total duration of 14 days. Any signs of toxicity and/or mortality were recorded during this observation period.

Phenyl-hydrazine-induced Anemia

Female Wistar rats were divided into five groups, as shown in Table 1, each consisting of six rats. All groups were provided with food and water ad libitum during the experimental period. The negative control was administered with a vehicle, while other groups were injected with PHZ (60 mg/kg) intraperitoneally in divided doses of 20 mg/kg for three days. The standard group was administered Orofer-XT (10 mg/kg) once daily for two weeks orally. The dose of Orofer XT was calculated by extrapolation from the human dose (100 mg of iron, twice daily). The test groups were administered 200 mg/kg and 400 mg/kg of extract orally, respectively. All oral administration was done using a soft rubber tube for gastric intubation without anesthesia. The treatment was continued for two weeks. The blood samples were collected into EDTA-coated tubes under mild pet ether anesthesia on the 1st, 3rd, 7th, and 14th days by the retro-orbital method. The collected blood samples were analyzed for hematograms (Jaiswal et al., 2014).

Adenine-induced CRF model of anemia

Female Wistar rats was randomly divided into five groups, as shown in Table 2, and treated for four consecutive weeks.

All groups received feed and water ad libitum. After 24 h of completion of treatment, the individual rat was anesthetized with mild pet ether anesthesia. The blood of anesthetized rats was withdrawn from the retro-orbital route and collected in the EDTA-coated tubes. Hematological and biochemical parameters were measured from collected blood samples (Ali et al., 2014).

Erythropoietin (EPO) assay

Serum EPO level was evaluated using a rat EPO ELISA kit (Krishgen Biosystems) following the procedure given by the manufacturer. The absorbance will be recorded at 450 nm using an ELISA microplate reader (Gheith et al., 2018).

Quantification of transferrin content

The transferrin content in the plasma of treatment and control group animals was estimated by the transferrin ELISA assay kit. (Krishgen Biosystems) The plasma will be separated and analyzed for transferrin content by the ELISA kit protocol. Samples, standards, and reagents will be prepared according to the protocol (Gheith et al., 2018).

Serum iron assay

Serum iron was estimated as per the method described by Stookey. To the blood sample 2.5 ml of hydroxylamine hydrochloride 220 mM in acetate buffer was added, pH 4.5. Iron gets separated from transferrin and reduced to ferrous from ferric. These ions react with ferrozine (50 µl, 16.7 mM) and form the violet-colored complex. The absorbance was measured at 560 nm wavelength (Stookey LL 1970).

Rota rod test

On day 1, animals were placed on the rotating rod with an adjusted speed of 20 rotations/min for 300 seconds. The two trials were conducted, and the animals that stayed on the rotating rod for 300 seconds during these successive trials were selected for this study. Animals were grouped into the Control group: which received normal saline solution; the standard group: which received standard drug Oxymetholone (Anadrol) 50 mg/kg; and the two test groups: which received BRAE at a dose of 200 mg/kg and 400 mg/kg, respectively, for seven days. On the seventh day, 45 minutes after the treatment, rats were placed on a rod rotating at a speed of 20 rotations per minute. The duration of staying on the rod of the individual animal was recorded with a cutoff time of 300 seconds (Dunham et al., 1957).

Grip-strength measurement

Kondziela's inverted screen method was used to measure grip strength. Animals were grouped into three groups: the control group received normal saline solution, the standard group received the standard drug oxymetholone (Anadrol) at 50 mg/kg, and the two test groups received BRAE at doses of 200 mg/kg and 400 mg/kg, respectively, for seven days. On day 1 and 14, after BRAE treatment, animals were placed on a wire mesh screen, and the screen was inverted. The time when an animal releases their hind limb and falls off was recorded (Deacon et al., 2013).

Data Analysis

The experimental results were expressed as the mean ± standard error mean (SEM). All the data were analyzed using analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the statistics software Prism graph pad.

RESULTS

Qualitative analysis

The percentage yield of the extract was found to be 5.4% w/w. Phytochemical evaluation of the SOAE showed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, and tannins.

In-vitro antioxidant activities

Diphenyl-1-picrylhydrazyl radical scavenging activity

In the photometric evaluation using DPPH assay, standard ascorbic acid showed 49.795 ± 0.69 % inhibition, while aqueous extract showed 45.94 ± 7.49 % of DPPH radical scavenging. The IC50 value of SOAE attained by DPPH was 13.05 ± 3.41 µg/ mL. The DPPH-scavenging effect of SOAE increased significantly when compared with standard ascorbic acid (Figure 1). This indicates its antioxidant activity.

HPTLC Fingerprinting

Chromatogram was developed for SOAE aqueous extract under a mobile phase saturated condition using mobile phase Ethyl Acetate: Water: Ethanol: Formic Acid (2: 7: 1: 3: 0.5 v/v/v). The HPTLC fingerprinting of SOAE aqueous extract showed the presence of 10 peaks with RF values for phenolic compounds (Figure 2.A) in the ascending order of 0.011, to 0.924 end values. The highest area (%) of the phytoconstituents was found to be 36.69 % and its corresponding RF value was 0.317. Also, the HPTLC fingerprinting of SOAE aqueous extract showed the presence of 10 peaks with RF values for flavonoid compounds (Figure 2.B) in the ascending order of 0.011, to 0.947 end values. The highest area (%) of the phytoconstituents was found to be 43.74 % and its corresponding RF value was 0.863. Exposure of the spotted and developed HPTLC plate at UV 254 nm showed the presence of light and dark bands. UV 366 nm exposure showed multi-colored bands of different intensities. This revealed the presence of polar and non-polar constituents in the aqueous extract. In the developed HPTLC fingerprinting, blue-colored bands indicated the presence of phenolic compounds, and yellow-colored zones indicated the presence of flavonoids in the SOAE aqueous extract.

Anti-anemic activity

Experimental protocol was approved by the IAEC of the Biocyte Institute of Research and Development, Sangli. (IAEC/Sangli/2022-23/01)

Acute toxicity study

During the two-week study, the toxicity signs were not observed, and there was no mortality recorded. As the doses used in the later study were 5–10 times smaller than the fixed dose used in the acute toxicity study, we can consider that the later study was conducted using safe doses.

Phenyl-hydrazine-induced Anemia

Induction of anemia in the participating group was done using the PHZ method. RBC count, hemoglobin content, and hematocrit percentage were determined in the rat blood sample collected in EDTA-containing vials using the MISPA VIVA (KT21092350) semi-auto analyzer.
SOAE, 400 mg/kg group, and standard group (Orofer XT 10 mg) showed significant activity ($P < 0.01$) in RBC count, HB level, and hematocrit% against the positive control group on the 14th day. [Table 3]

Adenine-induced CRF model of anemia

Administration of SOAE, 400 mg/kg group and the standard group (Orofer XT 10 mg) along with concurrent administration of adenine showed significant activity ($P < 0.01$) for hematological parameters against the positive control group in the adenine-induced CRF model. [Table 4]

Erythropoietin (EPO) assay, quantification of transferrin (Tf) content and iron binding assay

Daily administration of standard SOAE 200 mg/kg and 400 mg/kg along with concurrent administration of adenine showed dose-dependent improvement in EPO level and serum iron level (Figures 3 and 5).
Transferrin levels get increased in the positive control group while improved to normal level in the standard and test groups when compared with the negative control group (Figures 4).

Rota rod test

On seventh day, in the rota rod test, the standard drug and SOAE at doses of 200 mg/kg and 400 mg/kg showed a significant increase in time spent by the animals on the rotating rod when compared to the control group. SOAE at doses of 200 mg/kg and 400 mg/kg showed a dose-dependent increase in time spent on the roatating rod, that is, 61.83 ± 3.18 and 72.16 ± 3.30, respectively, when compared to the control group. Test group II spent more time on the rotating rod, indicating maximum muscle strength. The results of the rota rod test showed that SOAE extract significantly increased the motor coordination of the tested animals.

Grip strength test

The grip strength of the animals was measured both before (week 0) and after the administration of the SOAE (200 mg/kg and 400 mg/kg) at the end of week seven.
In grip strength measurement, the release limb time and drop-down time from the wire mesh in the standard and SOAE groups were significantly increased as compared to the control and standard groups. (Table 5 a and b)

DISCUSSION

Plant extracts consist of bioactive chemicals that support a variety of physiological processes and aid in preventing illness without having adverse consequences (Sevindik et al., 2017; Kumari et al., 2018; Chaves et al., 2020; Mohammed et al., 2021). According to the present study's preliminary examination, the extract includes phenolic compounds and flavonoids. We evaluated extract’s electron-donating capacity by employing the diphenyl-1-picrylhydrazyl test technique. This process depends on antioxidants' capacity to scavenge DPPH radicals, which causes the purple-colored DPPH solution to become transparent. The content and potency of the antioxidants in the extract are directly correlated with the degree of color change (Saeed et al.,

2012; Mohammed et al., 2020). The findings of the investigation revealed that SOAE possesses phytoconstituents with scavenging effects evaluated by DPPH method (Figure 1). These phytoconstituents can thus be utilized to promote wellness and prevent illnesses associated with oxidative damage. The findings of Idu et al. and the DPPH results were comparable. These authors used several scavenging assays to demonstrate the antioxidant properties of the herbal medicine *Mojeaga*. During erythrocyte formation, antioxidant molecules absorb excess reactive oxygen species (ROS), preventing oxidative damage to blood cells (Idu et al., 2022). The presence of phenolic and flavonoid content in SOAE was confirmed by HPTLC fingerprinting qualitative analysis (Figures 2 A and B). Antioxidant-rich fruits and vegetables include polyphenols that help lower oxidative stress (Sinaga FA 2020). The presence of flavonoids and phenolic compounds in the SOAE provides evidence for the antianemic action and influence on muscular coordination.

Low erythrocyte counts and decreased hemoglobin levels are the hallmarks of anemia. Hypoxia results from this lack of oxygen (Lee et al., 2014). Phenyl hydrazine is used for generating anemia in laboratories. PHZ causes hemolytic anemia and raises reactive oxygen species and lipid peroxidation (Berger, 2007). Results shown that after PHZ therapy, SOAE at a dosage of 400 mg/kg significantly increases hematocrit, hemoglobin concentration, and red blood cell count (Table 3). In this investigation, we also evaluated the effect of SOAE on biochemical and hematological parameters in the model of adenine-induced CRF. Because renal function declines quickly in inflammatory kidney injury, iron deficiency anemia is frequently seen. Impaired renal function results into decreased synthesis of erythropoietin, which is essential for erythropoietin (Nangaku et al., 2006). The liver produces and releases the protein transferrin, which is necessary for the blood's transportation of iron. To improve the body's use of the iron that is available, transferrin levels rise during anemia (Adams et al., 2011).

The hematological and biological parameters improved in a dose-dependent manner with SOAE, in adenine induced model (Table 4). Not much study has been done in the past about how SOAE affects biochemical markers like Tf and EPO. Our findings in relation to these parameters suggest that SOAE protects renal tissues. SOAE maintained the kidney's capacity to promote erythropoiesis by synthesizing erythropoietin (Figure 3). Additionally, iron content and serum transferrin level were restored to normal level by SOAE (Figures 4 and 5).

Hematocrit, hemoglobin count, and erythrocytes are all reduced during endurance exercise. Athletes frequently experience these alterations (Damian et al., 2021), which reduce their stamina and physical performance and cause lethargy (Chatard et al., 1999). Previous studies have demonstrated that natural products can prevent or minimize fatigue and improve athletic performance without having any adverse impacts (Kicman, 2008). This study demonstrated that SOAE has a significant effect on models of muscular coordination. Increased time spent on the rotating rod and improved grip strength were the outcomes of administering SOAE at 200 mg/kg and 400 mg/kg dosages (Tables 5 a and b). According to this, oral SOAE treatment improved muscle coordination and had anti-anemic effects. These findings align with a study conducted by Khot VS and Kumbhar ST. (Khot VS 2023)

CONCLUSION

Natural compounds have been more and more in demand in recent years due to their safety and efficacy as well as the variety of chemical elements they contain. Improved phytochemical profile, antioxidant properties, and therapeutic potential were demonstrated by SOAE in relation to anemia and anemia-induced general debility. These results demonstrate the extract's diverse nutritional and health-promoting qualities, which affect physiological factors associated with muscle function in addition to treating anemia.

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Table 1. Phenyl Hydrazine Induced Anaemia (n=6)

Sr No.	Name of Group	Treatment	Oral Doses, frequency	Blood withdrawal
1	- Ve Control	Vehicle	0.5ml, daily	Retro orbital sinus, 0.5-1mL on 1st, 3rd,7th and 14th day using Pet. Ether.
2	+ Ve Control	Vehicle + PHZ	60mg/Kg, 20mg 3 days	
3	Standard	Orofer-XT+ PHZ	10 mg/kg, once daily + PHZ 20 mg 3 days	
4	SOAE 200 mg/kg	SOAE + PHZ	200 mg/Kg, once daily + PHZ 20 mg 3 days	
5	SOAE 400 mg/kg	SOAE + PHZ	400 mg/Kg, once daily + PHZ 20 mg 3 days	

Note: Except –ve control, all groups were administered with *PHZ* 20mg /Kg intra-peritoneally as inducer for 3 days.

-Ve Control: Normal group, +Ve Control: Diseased group, SOAE: *S. oleracea* aqueous extract.

Table 2. Adenine induced CRF model of anaemia (n=6)

Sr No.	Name of Group	Treatment	Oral Doses, frequency	Blood withdrawal
1	- Ve Control	Vehicle	0.5ml, daily	Retro orbital sinus, 0.5-1mL on 1st, 3rd,7th and 14th day using Pet. Ether.
2	+ Ve Control	Vehicle + adenine	0.75 %, w/w, in feed	
3	Standard	Orofer-XT+ adenine - 0.75 %, w/w, in feed	10mg/kg, daily + 0.75 %, w/w, in feed	
4	SOAE 200 mg/kg	SOAE + adenine - 0.75 %, w/w, in feed	200 mg/Kg, once daily + 0.75 %, w/w, in feed	
5	SOAE 400 mg/kg	SOAE + adenine - 0.75 %, w/w, in feed	400 mg/Kg, once daily + 0.75 %, w/w, in feed	

Note: Except –ve control, all groups were administered with adenine 0.75% through feed as inducer for 14 days.

-Ve Control: Normal group, +Ve Control: Diseased group, SOAE: *S. oleracea* aqueous extract.

Table 3. Effect of SOAE aqueous extract on Red blood cell count, Hemoglobin count and Hematocrit % in phenylhydrazine induced anemia.

Group	RBC count (lacs/mm3)				Hemoglobin count g/dL				HCT count %			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
- Ve Control	64.0 ±5.0	63.4 ±6.7	62.3 ±2.8	64.6± 4.2	13.3 1± 0.34	12.6 8± 0.42	12.8 9± 0.12	13.2 7± 0.18	38.1 2± 1.21	41.4 2± 1.67	39.3 3± 1.78	40.79 ± 1.34
+ Ve Control	65.8 ±4.3	31.8 ±8.7	39.5 ±4.5	41.9± 8.3	13.0 8± 0.46	6.35 ± 0.28	6.92 ± 0.34	7.23 ± 0.35	39.8 9± 2.63	24.3 1± 2.04	27.2 4± 1.56	30.29 ± 1.45
Standard	68.0 ±6.7	39.1 ±5.6	58.9 ±6.7	69±3. 5**	12.9 3± 0.35	8.14 ± 0.31	11.4 6± 0.41	14.2 3± 0.18 ***	40.1 8± 1.67	23.4 8± 1.45	33.6 7± 2.03	41.23 ± 1.56**
SOAE 200 mg/kg	66.7 ±0.4 2	35.6 ±0.2 3	47.9 ±0.4 5	65.9± 0.26**	13.5 6± 0.28	7.93 ±0.6 3	10.1 3±0. 25	11.8 9±0. 34*	40.4 2±2. 12	22.8 4±2. 56	32.4 5±1. 65	38.68 ±2.05 *
SOAE 400 mg/kg	64.6 ±0.3 8	38.9 ±0.7 3	52.3 ±0.3 4	72.2± 0.62**	13.4 5±0. 27	8.12 ±0.4 9	11.4 5±0. 27	13.7 8±0. 25**	41.2 3±1. 56	23.4 5±1. 56	33.0 5±1. 42	40.64 ±1.45 **

SOAE: S. oleracea Aqueous Extract, RBC: Red blood cell, HCT: Hematocrit.

Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with negative control *P<0.05, ** P<0.01.

Table 4. Effect of SOAE aqueous extract on Red blood cell count, Hemoglobin count and Hematocrit % in Adenine induced CRF model of anemia.

Group	RBC count (lacs/mm3)				Hemoglobin count g/dL				HCT count %			
	Day 1	Day 9	Day 18	Day 28	Day 1	Day 9	Day 18	Day 28	Day 1	Day 9	Day 18	Day 28
- Ve Control	65.6 ±0.5 0	61.9 ± 0.45	61.0± 0.21	65.8± 0.31	12.3 4±0. 24	12.6 8±0. 12	12.5 4±0. 56	12.1 7±0. 42	43.0 1±1. 21	42.4 3±1. 43	41.85 ±2.13	41.45 ±1.54
+ Ve Control	61.3 ±0.4 3	40.3 ±0.8 7	42.3± 0.35	46.8± 0.43	13.1 2±0. 46	8.54 ±0.1 8	8.67 ±0.3 4	9.56 ±0.1 5	41.9 1±2. 15	26.4 2±1. 74	28.14 ±1.56	31.32 ±1.45
Standard	68.0 ±0.6 7	38.7 ±0.4 6	56.7± 0.53	65.4± 0.46**	12.1 5±0. 25	8.24 ±0.2 1	11.5 6±0. 15	13.2 4±0. 18**	41.3 2±1. 43	26.6 5±1. 65	33.46 ±1.33	41.12 ±1.78 **

								*				
SOAE 200 mg/kg	62.6 ±0.2 5	37.5 ±0.5 2	44.5± 0.55	60.8± 0.64* *	13.3 6±0. 13	7.23 ±0.1 3	10.1 5±0. 45	11.4 9±0. 41*	40.4 6±2. 34	22.6 2±1. 86	33.35 ±1.85	39.68 ±1.67 *
SOAE 400 mg/kg	67.6 ±0.7 6	35.6 ±0.4 3	55.6± 0.76	65.3± 0.64* *	13.6 3±0. 37	8.35 ±0.5 2	11.3 5±0. 47	12.8 ±0.1 4*	40.9 3±2. 16	23.3 2±1. 36	32.54 ±1.42	41.3± 1.15* *

SOAE: S. oleracea Aqueous Extract, RBC: Red blood cell, HCT: Hematocrit.

Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with negative control *P<0.05, ** P<0.01.

Table 5. Effect of SOAE on limb release by grip strength measurement

a) Release Limb

Group	**Release limb time before**	**Release limb time after**
I. Normal control	68±4	61±3
II. Standard	51±6	53±8
III. SOAE 200 mg/kg	62±2	64±6
IV. SOAE 400 mg/kg	69±4	74±2`

Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with control *P<0.05, ** P<0.01.

Effect of SOAE on drop down by grip strength measurement

b) Drop Down

Group	**Rat falling time before**	**Rat falling time after**
I. Normal control	2.4±0.4	2.3±0.2
II. Standard	3.1±0.2	3.14±0.4
III. SOAE 200 mg/kg	3.3±0.5	3.6±0.2
IV. SOAE 400 mg/kg	3.1±0.34	4.2±0.5`

Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with control *P<0.05, ** P<0.01.

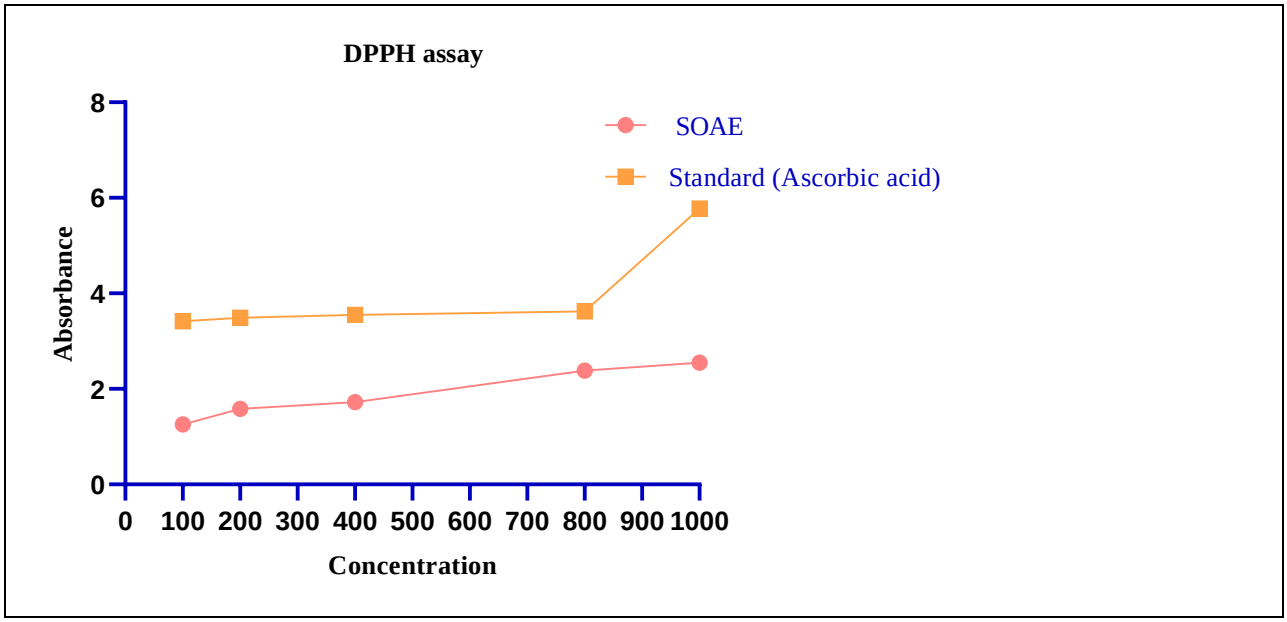

Figure 1. DPPH scavenging assay of SOAE compared with standard ascorbic acid. Results were expressed as Mean ± SEM.

A. B.

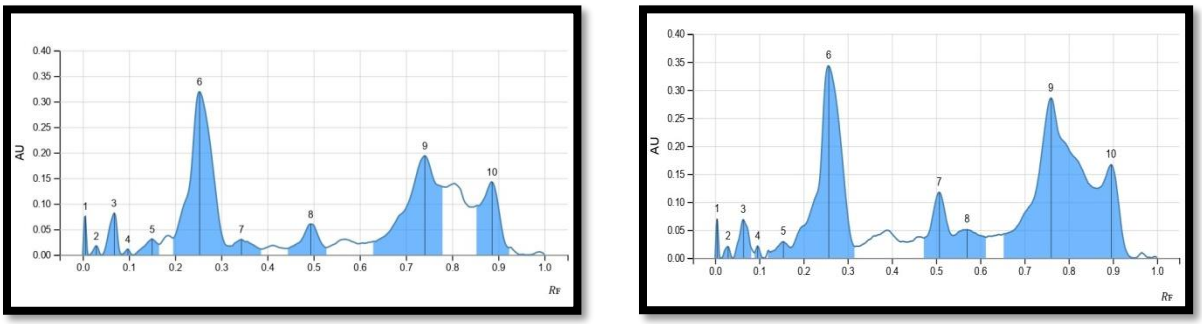

Figure 2.HPTLC fingerprint of SOAE revealing presence of (A) 10 phenolic compounds & (B) 10 flavonoids.

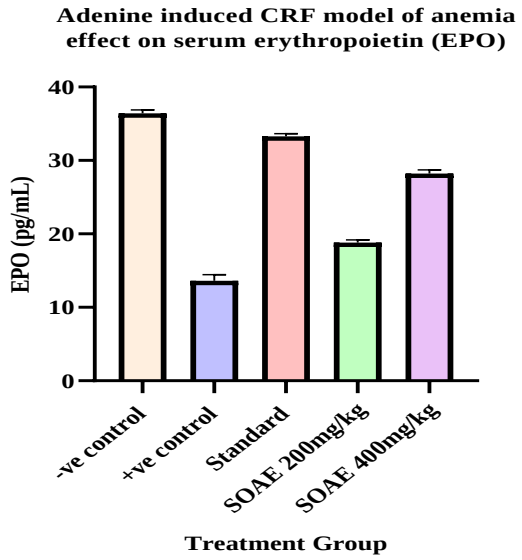

Figure 3. Effect of SOAE aqueous extract on erythropoietin level in Adenine induced CRF model of anemia. Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with negative control *P<0.05, ** P<0.01.

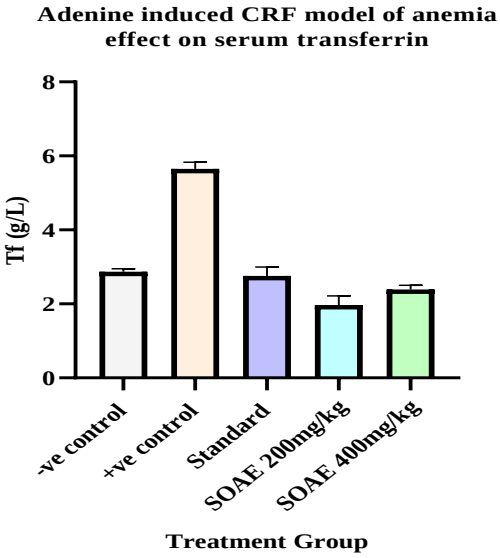

Figure 4. Effect of SOAE aqueous extract on transferrin level in Adenine induced CRF model of anemia. Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with negative control *P<0.05, ** P<0.01.

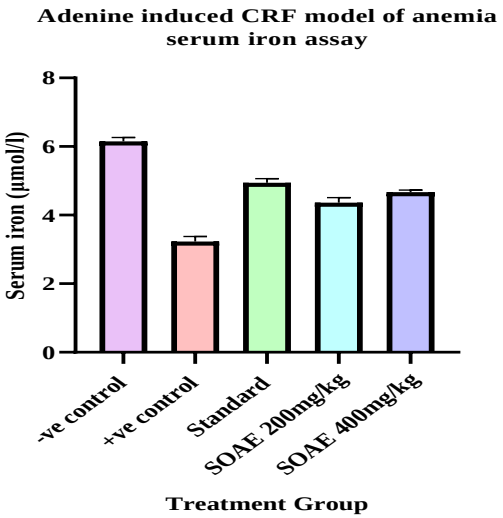

Figure 5. Effect of SOAE aqueous extract on serum iron level in Adenine induced CRF model of anemia. Values are expressed as mean ± SEM. (n=6) Difference in variance analyzed amongst groups using one-way ANOVA and Dunnett's t as post-test. Difference in variance amongst groups when compared with negative control *P<0.05, ** P<0.01.