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Haematinic Potential of *Jussiaea repens* L – A Search for Antianaemic Herb

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Authors' contributions

This work was carried out in collaboration among the authors. Authors NP and IC proposed the study design and supervised the work. Authors SG and AD carried out the research work including animal maintenance, drug preparation and experiments. All authors read and approved the final manuscript.

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ABSTRACT

Background: Anaemia is a common nutritional disorder. Different harmful synthetic drugs are used to combat it. So herbal remediation is in demand.
Aims: In this study a common medicinal herb *Jussiaea repens* (JR) was used. It is traditionally used to prevent diabetes, diuretics, fever, cough etc. But no report yet is available on anaemia prevention.
Methodology: 30 adult male albino rats were grouped as control (n=6), 2,4-DNPH induced anaemia (2 mg/100 gm body weight/day i.p.,n=18) and JR treatment (20 mg/100 gm body weight/day orally, n=6). After 7 days, 6 anaemic animals were killed, 6 kept as DNPH withdrawal and other 6 as JR supplement followed by 2,4-DNPH withdrawal. All animals were maintained till 21st day of treatment.
Results: Body growth rate was significantly (P<0.001) decreased in 2,4-DNPH induced anaemic group which was partially recovered in withdrawal and JR supplement. Liver weight was significantly decreased (P<0.01) in anaemic group than control but was significantly increased

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(P<0.01) in JR treatment. Spleen weight was significantly increased (P<0.05) in anaemic group but significantly decreased (P<0.05) in JR supplement and treatment. Significant decrease (P<0.05) in adrenal weight was found after 2,4-DNPH withdrawal, where other organs remain unchanged. The anaemic group showed insignificant reduction in total erythrocyte count and significant reduction (P<0.05) in haemoglobin concentration than control and were significantly recovered (P<0.05) after JR supplementation and treatment. PCV was significantly higher in withdrawal (P<0.05) and JR treatment (P<0.01) than anaemic group, where MCHC was significantly higher (P<0.01) only in JR treatment. But no remarkable change observed in MCH and total leukocyte count. Free haemoglobin in plasma showed significant rise (P<0.005) in anaemic group, but was significantly reduced alike MCV after 2,4-DNPH withdrawal, JR supplementation and treatment, which supported higher osmotic fragility of RBC in anaemic group. SEM observation of erythrocyte morphology also supported it.

Conclusion: So, crude ethanolic extract of JR can prevent anaemia and can be used further as potent antianaemic drug.

Keywords: Jussiaea repens; haematinic; 2,4-dinitrophenylhydrazine; anaemia; haemoglobin; osmotic fragility.

1. INTRODUCTION

Anaemia is the most common and widespread nutritional disorder in the world. The incidence of anaemia is higher in third world countries. The World Health Organization (WHO) classified anaemia as a severe public health problem (prevalence >40%) for children under five in 69 countries and for pregnant women in 68 countries [1]. According to National Family Health Survey (NFHS-3), the incidence of anaemia in urban population is 71%, in rural areas it is 84% and the overall incidence is 79% [2]. Anaemia is manifested by tiredness, anorexia, inability to concentrate, somnolence, reduced immunity, poor schooling performance and decreased quality of life [3]. There are over 400 types of anaemia, many of which are rare but in all cases the circulating erythrocytes are below normal or their oxygen-carrying capacity is insufficient to meet the physiologic needs [4]. Anaemia exists with number of causes, such as malaria, parasitic infection, nutritional deficiencies, drug toxicity as well as genetic or acquired defect [5-7]. To prevent anaemia, man consumes varieties of synthetic drugs having dreaded side effects [8], where herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. In China for instance, blood diseases such as malformation of blood circulatory system, anaemia, varicose veins and haemorrhages have been treated with plant materials [9].

Nowadays researchers are trying to develop different drugs with therapeutic uses from plant extracts. Researchers find that medicinal plant and their individual constituents act in similar fashion as the modern drugs and sometime better without any side effects. Such a medicinal plant, *Ludwigia adscendens* L. (Synonym – *Jussiaea repens* L.; family - onagraceae) is a herb, commonly known as creeping water prime rose. *Jussiaea repens* L., locally known as 'Kesardam' in different parts of India. It is a well known medicinal plant, found in wetlands. It grows in fresh water, ponds, canals of roadside and wetlands. It has been found in different districts of West Bengal, Jharkhand, Orissa and Manipur in India as well as in China, Africa, Thailand, Malaysia, Australia, New Guinea and Philippines at low and medium altitudes. By chromatographic, chemical and spectroscopic studies, different scientists reported that aerial parts of this plant is composed of different metabolites like rutin, kaempferol, quercetrin, quercetin, terpenes, triterpenes, trifolin, trifolin 2″-O-gallate, hyperin, hyperin 2″-O-gallate, guaijaverin, reynoutrin, juglanin, avicularin etc. [10,11]. A new acylated avicularin, namely avicularin 2″-(4‴-O-n-pentanoyl)-gallate along with these metabolites have also been isolated from the ethyl acetate extract of the aerial parts of *Jussiaea repens* L. [12]. Pharmacologists reported the clinical uses of this plant as hepatoprotective, antidiabetic, antihelmintic, antidysenteric, antiinflammatory, antibacterial, antigonadal and antifertile property and have fibrinolytic activity [12-14]. It has also some therapeutic uses i.e. in ulcer, fever, cough, diuretic, urinary tract infection etc. [15]. Inspite of different therapeutic uses of *Jussiaea repens,* it may be used for the treatment of anaemia like other medicinal herbs [16-20], but its effect on haematological parameters are still lacking. So,

in present study, an attempt has taken to see whether the crude ethanolic extract of *Jussiaea repens* has any effect on blood parameters or whether it play any protective role in 2,4-DNPH induced anemia and can be used as antianemic drug in future.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant *Jussiaea repens* L. was collected from wetlands of 24 Parganas (N), West Bengal, India, during the month of March - April. The material was identified and authenticated by taxonomist of Central National Herbarium (Kolkata), Botanical Survey of India (BSI), Shibpur, Howrah, West Bengal, India having voucher specimen number NP-01 dated 25.03.2011. Fresh plants were carefully washed under running tap water and then with distilled water, air dried at 35-40°C for 4-5 days, homogenized to a coarsely powder by mixer grinder and stored for extraction.

2.2 Extract Preparation

200 gm coarsely powdered plant of *Jussiaea repens* L. was extracted with 2 liters of 70% ethanol by continuous hot extraction method using soxhlet apparatus for 8 hours and filtered. The ethanolic extract was concentrated using rotary evaporator at 60°C and was finally dried at 40°C with the help of heating mantle. Finally yield 8% solid brown extract and was stored in an air tight container at 4°C for further use in experiments [21].

2.3 Animal Selection and Maintenance

Thirty adult male albino rats (*Rattus norvegicus* L. of Wistar strain) weighing 130±10 gm were selected for the experiment and kept under specific pathogen free conditions. The rats were maintained under standard laboratory condition (temperature 25±2°C, 12/12hr. dark and light, relative humidity 40-60%) with free access to standard normal diet, prescribed by ICMR, NIN, Hyderabad, India and water *ad libitum* [22]. Animals in each group were housed in clean and large polypropylene cages. The animals were acclimatized to the laboratory condition for a period of one week before starting the experiment. All efforts were made to minimize the suffering of animals.

2.4 Experimental Design and Drug Administration

Animals were randomly divided into five groups of six animals each (n=6), as –

- Group I - given 0.1 ml distilled water/100 gm b.wt./day for first 7 days through intraperitoneal (i.p.) and from 8th day to 21st day through oral feeding.
- Group II - given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p.
- Group III - given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p. and 0.1 ml distilled water/100 gm b.wt./day from 8th day to 21st day through oral feeding.
- Group IV - given 2 mg 2,4-DNPH/100 gm b.wt./day for first 7 days through i.p. and 20 mg extract/100 gm b.wt./day from 8th day to 21st day through oral feeding.
- Group V - given 0.1 ml distilled water/100 gm b.wt./day for first 7 days through i.p. and 20 mg extract/100 gm b.wt./day from 8th day to 21st day through oral feeding.

Artificial anaemia in group II was induced by administering 2,4-DNPH considering the haemoglobin level less than 13 gm/dl as anaemia [23,24].

2.5 Animal Sacrifice

Rats were sacrificed between 10-12am, under light ether anaesthesia, on the day 24 hours after cessation of treatment. Animals of group II was sacrificed on 8th day and others on 22nd day.

2.6 Blood and Tissue Collection

Blood was collected from hepatic vein of rats after sacrifice. About 3 ml blood was dispensed into specimen vials containing anti-coagulant EDTA (ethylene diamine tetra-acetic acid). Different organs like heart, lungs, liver, kidney, spleen and adrenal glands were dissected out, trimmed, blotted and weighed immediately. Plasma was isolated by centrifuging the anti-coagulated blood in 3000 rpm for 5 minutes. Tissues and blood samples were kept in 4°C for further experiments.

2.7 Determination of Haematological Indices

The whole blood with anticoagulant was used for assay of haematological parameters, i.e. total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb) concentration, Packed Cell Volume(PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) by following methods –

a) Total erythrocyte count (TEC) – was estimated using improved Neubauer counting chamber [25].
b) Total leukocyte count (TLC) – was estimated using improved Neubauer counting chamber [25].
c) Hb (gm/dl) – was determined by the cyanomethaemoglobin method [26].
d) Free haemoglobin in plasma (gm/dl) - was determined by the cyanomethaemoglobin method [26].
e) PCV (Packed Cell Volume) – with Wintrobe haematocrit tubes [25].
f) MCV, MCH and MCHC were calculated as –
    i. MCV (cubic microns) = (PCV x 10) / RBC(in million/mm^3)
    ii. MCH (picograms) = (Hb in gm/dl x 10) / RBC(in million/mm^3)
    iii. MCHC (gm/dl) = (Hb in gm/dl x 100) / PCV

2.8 Measurement of % Haemolysis by Isotonic Saline

Haemolysis was determined by measuring haemoglobin released from red blood cells relative to the total cellular haemoglobin content. 10 µl of fresh blood was added to 5 ml normal saline and then incubated at 37ºC for 30 min. After centrifugation (3000 rpm for 5 min), supernatant was aspirated and absorbance was measured at 540 nm. The % haemolysis was taken against complete haemolysis. It can be expressed as:

$$\%\ haemolysis = \left(\frac{A_{sample}}{A_{100\%\ lysis}}\right) \times 100$$

Where A_{sample} and $A_{100\%lysis}$ are the absorbance of haemoglobin released from red blood cells in normal saline and after complete haemolysis after incubation in distilled water [27,28].

2.9 Osmotic Fragility Test

Fresh blood was added to the different hypotonic saline (pH 7.4) ranging from 0–0.9% (0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.9%) in the ratio of 1:100 respectively. Then the mixtures were gently shaken, incubated at room temperature for 30 min and centrifuged to precipitate the non-haemolized RBC. Osmotic lysis of RBC was determined by the release of haemoglobin into extracellular fluid. Absorbance of the supernatant was measured at 540 nm using a spectrophotometer. The % haemolysis against each NaCl concentration relative to the blank salt concentration was calculated [29,30].

2.10 Scanning Electron Microscopy (SEM) Study

1 drop of blood sample was taken in a cover slip and thin smear was drawn. The suspension was fixed by 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2h at room temperature before drying. Preparation was dehydrated by graded alcohol and air dried, coated with gold and finally observed under S-530 Hitachi SEM [31].

2.11 Statistical Analysis

The recorded values were expressed in mean±SEM. The control group and experimental groups were compared to each other by using one way ANOVA with post hoc Tukey's multiple comparison test. The tests were performed using Graph Pad Prism version 6.05 software. The value of *P<0.05* was considered to be statistically significant.

3. RESULTS

3.1 Body Growth Rate

Body growth rate was considered in 2 phases, one is 1^{st} -7^{th} day and other is 8^{th}-21^{st} day of treatment. The treatment of 2,4-DNPH (group II, III and IV) at a dose of 2mg/100gm b.wt./day for first consecutive 7days caused significant reduction in body growth rate in compare to vehicle control (group I and V). In next 14 days (8^{th}-21^{st} day) body growth rate in group III was recovered but was significantly lower than group I, where it was significantly higher in group V in compare to group III.

3.2 Relative Organ Weights

In group II, liver weight (3.184±0.094 gm) was reduced and spleen weight (0.389±0.044 gm) was increased significantly in respect to group I

(liver weight 3.898±0.143 gm and spleen weight 0.269±0.013 gm), whereas opposite effect was shown in group V (liver weight 3.954±0.165 gm and spleen weight 0.280±0.015 gm) when compared to group II. Spleen weight in group IV (0.268±0.019 gm) and adrenal glands weight in group III (0.019±0.001 gm) were recovered towards normal significantly in respect to group II (adrenal weight 0.025±0.001 gm). Relative weights of heart, lungs and kidney showed no significant changes among the groups. Results of relative organ weight are showed in Fig. 1.

3.3 Haematological Parameters

Hb was significantly decreased and free Hb in plasma was significantly increased in group II in compare to group I. But the Hb concentration was significantly increased in group V than group III, where free Hb in plasma in group III, group IV and group V were significantly reduced than group II. TEC was decreased in group II than control but was significantly increased in group III, group IV and group V in compare to group II. Hb in group IV and group V, PCV in group III and group V and MCHC in group V showed significant increase when compare to group II. The MCV in group III, group IV and group V was significantly lower than group II which was significantly increased than group I. Data of MCH showed no significant change (Table 2).

3.4 Percent (%) Haemolysis Test

In Fig. 2(A), % haemolysis was significantly higher in group II (4.589±0.265) and III (2.095±0.106) in respect to group I (1.313±0.064), whereas it was significantly reduced in group III, IV (2.059±0.219) and V (1.210±0.078) when compared with group II. % haemolysis in Group V was also significantly lower than group III and IV.

Table 1. Percent changes in average body growth rate in 2 phases of drug treatment among the groups

Groups	1st day – 7th day	8th day – 21st day
Group I (Control)	+ 3.421±0.445	+ 7.662±0.775
Group II (2, 4-DNPH)	- 6.208±0.937$^{a,b\#}$	Sacrificed on 8th day
Group III (2,4-DNPH withdrawal)	- 6.008±0.833$^{a,b\#}$	+ 3.904±0.816^{a*}
Group IV (Extract after 2,4-DNPH withdrawal)	- 5.990±0.882$^{a,b\#}$	+ 5.721±0.938
Group V (Extract alone)	+ 3.623±0.461	+ 8.207±0.691^{c**}

*Values are expressed as mean±SEM, n=6. P<0.05 is considered as significant. Values bearing superscripts are significantly different (*P<0.05; **P<0.01; #P<0.001; a = in respect to Group I; b = in respect to group V; c = in respect to group III)*

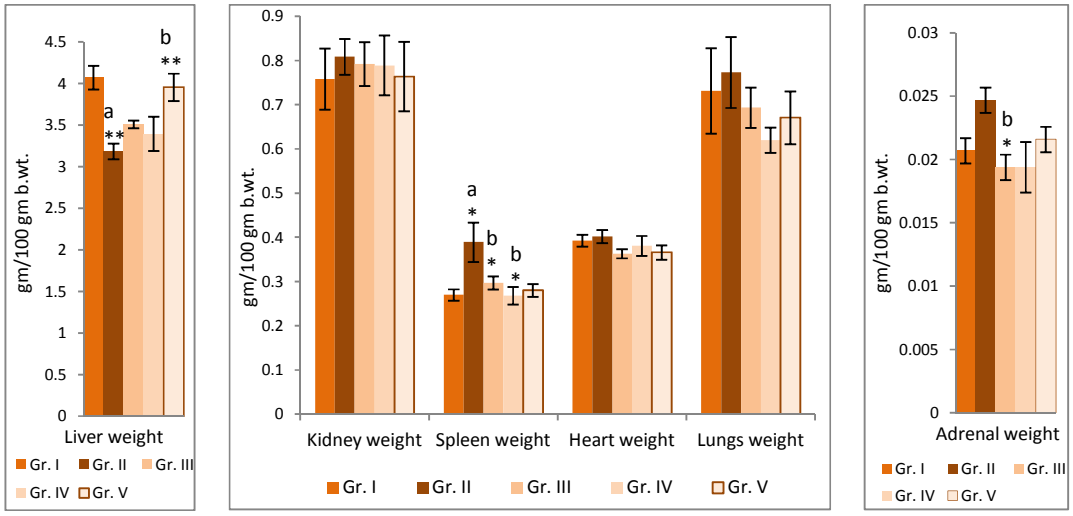

Fig. 1. Relative weights (gm) of vital organs in different groups

*Values are expressed as mean±SEM, n=6. P<0.05 is considered as significant. Values bearing significantly differences *P<0.05; **P<0.01; a = in respect to Group I; b = in respect to group V.*

Key: Gr. I = control; Gr. II = 2,4-DNPH(2 mg/100 gm); Gr. III = 2,4-DNPH(2 mg/100 gm)+withdrawal, Gr. IV = 2,4-DNPH(2 mg/100 gm)+JR(20 mg/100 gm); Gr. V = JR(20 mg/100 gm); b.wt. = body weight

3.5 Osmotic Fragility Test

Mean percentage of haemolysis at varying NaCl concentration is shown in Fig. 2(B). In 0.4 gm% NaCl concentration, the % haemolysis in group I,II,III,IV and V were 22.61%, 70.09%, 65.19%, 52.88% and 32.26% respectively, where in 0.5 gm% NaCl which were 5.90%, 37.47%, 26.07%, 21.69% and 8.69%. So, osmotic fragility test of erythrocytes showed that osmotic fragility was higher in group II than other groups and was recovered in JR supplement and treated group.

3.6 SEM Study

The changes in topography of rat erythrocytes in all groups were studied by scanning electron microscope (SEM). It was observed that control animals in group I had typical appearance of erythrocytes where most cells were typical discocytes. Erythrocytes in rats treated with 2,4-DNPH in group II showed different stages of echinocytes (with blebs and protuberances on their surfaces), achantho-echinocytes (characterized by blebs, spicules of varying length, irregularly distributed over the entire cell surface with knobby ends) and atypical shape (irregularly outlined with sharply changed topography) were also found. This effect was partially recovered towards control in group IV than group III without acanthocytes having peripheral protuberences.

Table 2. Changes in different haematological parameters in vehicle control and experimental group of rats

Parameters	Group I (control)	Group II (2,4-DNPH)	Group III (withdrawal after 2,4-DNPH)	Group IV (extract after 2,4-DNPH)	Group V (extract only)
TEC (million/ mm^3)	8.650±0.475	6.831±0.389	9.440±0.650^{b*}	9.515±0.622^{b*}	10.920±0.581$^{b•}$
TLC (x10^3/ mm^3)	6.586±0.505	8.014±0.379	6.191±0.440	6.568±0.968	7.063±0.467
Hb (gm/dl)	14.050±0.576	11.250±0.437^{a*}	13.700±0.839^{c*}	13.830±0.657^{b*}	16.330±0.517$^{b\#}$
PCV (%)	47.730±0.862	43.020±0.709	48.470±1.243^{b*}	46.780±2.058	50.760±0.899^{b**}
MCV (fl)	55.830±2.453	63.720±2.657	52.180±2.491^{b*}	49.800±2.360^{b**}	47.060±2.321$^{b•}$
MCH (pg)	16.310±0.259	16.560±0.338	14.660±0.771	14.680±0.559	15.140±0.829
MCHC (gm/dl)	29.390±0.806	26.110±0.613	28.250±1.552	29.550±0.313	32.260±1.329^{b**}
Free haemoglobin in plasma (gm/dl)	0.265±0.047	0.547±0.049$^{a•}$	0.357±0.035^{b*}	0.353±0.025^{b*}	0.208±0.026$^{b\#}$

*Values are expressed as mean±SEM, n=6. P<0.05 is considered as significant. values bearing superscripts are significantly different (*P<0.05, **P<0.01, •P<0.005, #P<0.001, a = in respect to Group I; b = in respect to Group II; c = in respect to Group V). Key: TEC=total erythrocyte count; TLC=total leukocyte count; Hb=haemoglobin; PCV=packed cell volume; MCV=mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=mean corpuscular haemoglobin concentration; 2,4-DNPH=2,4-dinitrophenylhydrazine*

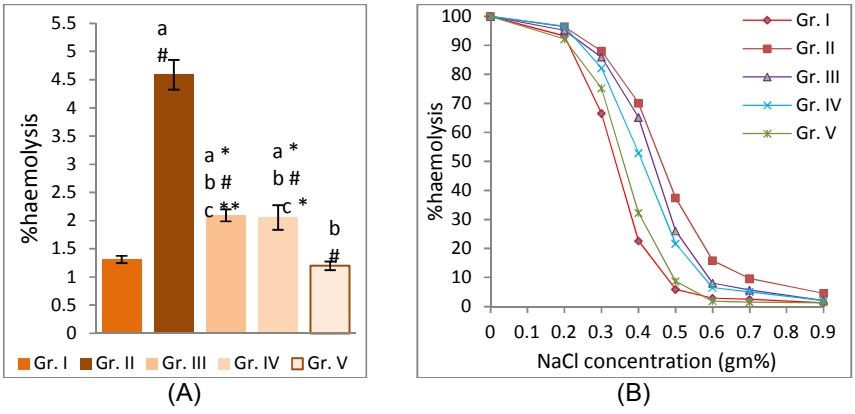

Fig. 2. % haemolysis and osmotic fragility test in different experimental groups

*(A) % haemolysis at isotonic saline in different groups (*P<0.05, **P<0.01, #P<0.001, a = in respect to Group I; b = in respect to Group II; c = in respect to Group V) Key: Gr. I = control; Gr. II = 2,4-DNPH(2 mg/100 gm); Gr. III = 2,4-DNPH(2 mg/100 gm)+withdrawal, Gr. IV = 2,4-DNPH(2 mg/100 gm)+JR(20 mg/100 gm); Gr. V = JR (20 mg/100 gm), (B) Fragility curve obtained from the experimental data showing % haemolysis versus NaCl concentration*

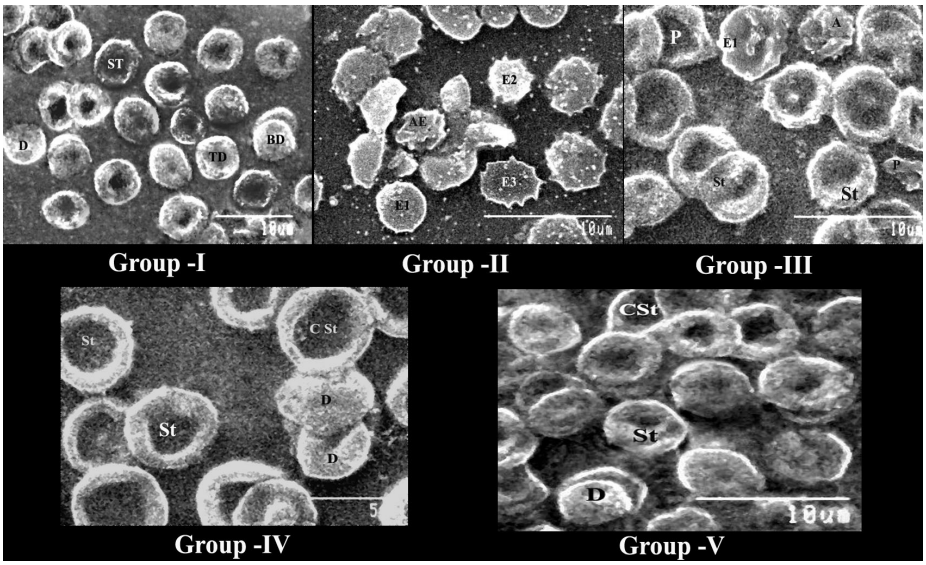

Fig. 3. Scanning electron micrograph of erythrocytes in control and different drug treated groups showing Biconcave Discoytes (BD), typical biconcave discoytes (TD), discoytes (D), Cup shaped stomatocytes (CSt), stomatocytes (St), echinocytes stage I (E1), echinocytes stage II (E2), echinocytes stage III (E3), Echinocytes (E), Acantho –echinocytes (AE), acanthocytes (A), peripheral protuberances (P)

4. DISCUSSION

Anaemia is a worldwide nutritional threat to human beings. The primary causes of anaemia are nutritional deficiencies and haemolysis. In spite of broad classification, the decrease of total RBC count and hemolytic anaemia are very common. To prevent anaemia, different synthetic drugs are commonly used by the people which are mostly hazardous [8]. So, herbal haematinics are mostly preferred nowadays in different countries like China [9]. So, to develop herbal hematinics, artificial anaemia was induced in rats by 2,4-DNPH, a phenylhydrazine (PHZ) derivative administration [24].

In the present study, the significant reduction in body growth rate was observed in first seven days treatment in artificial anaemic groups was mostly due to 2,4-DNPH induced oxidative stress, linked to reduced disaccharidases activity in rats [18,20,32]. The reduced liver weight in 2,4-DNPH induced group is due to hepatotoxicity and cirrhosis of liver caused by hepatic cell atrophy and liver DNA fragmentation as one of the major complications of anaemia. Again the rise of splenic weight in 2,4-DNPH treated group might be due to deposition of blood pigments and partially destroyed erythrocytes in the spleen [33,34]. In anaemic group, the weight of adrenal gland was increased than other groups. It was possibly due to 2,4-DNPH induced stress, which causes stimulation of ACTH secretion as a result of oxidative stress [35]. The decreased body weight causes hyperactivity of adrenal gland causing increased secretion of adrenaline followed by increased glucose uptake and lipogenesis that finally balances body weight [36]. According to WHO report, the PHZ is “readily biodegradable” and its elimination was 77% after 10 days and 97% after 28 days. A significant proportion of a single dose was excreted relatively slowly, 50% of the dose was excreted within 4days of dosing. No sufficient data regarding PHZ accumulation in tissues on repeated exposure yet are available [33]. So, in this study withdrawal of 2,4-DNPH caused partial recovery in body growth rate and relative weights of different organs, whereas treatment of *Jussiaea repens* L. (JR) extract alone and in 2,4-DNPH withdrawal rats caused rapid recovery expressing the possible protective action against oxidative stress, which is similar to other different studies [17-20].

In haematological studies, 2,4-DNPH induced anaemia in group II caused remarkable reduction in TEC and Hb% which were significantly recovered in 2,4-DNPH withdrawal and JR supplemented groups. PCV and MCHC also showed reductive changes in anaemic group where MCV was significantly higher. But MCH

remained unaltered. This observation indicated that 2,4-DNPH somehow inhibits erythropoiesis possibly by inducing micronucleated polychromated and hypochromic erythrocytes [37]. Pandey et al. in 2014 also reported that PHZ blocks erythropoietin receptors causing anaemia [34]. Estimation of free Hb in plasma and osmotic fragility test showed marked correlative rise in 2,4-DNPH induced anaemic group.

PHZ and 2,4-DNPH induces lysis of RBC by formation of toxic free radicals which can attack haemoglobin causing oxidative damage and formation of 'Heinz Bodies' [24]. Phenylhydrazine interacts with haemoglobin and cytochrome P-450 in an oxidation reaction resulting in the generation of destructive free radicals, which are responsible for subsequent haemolysis [33]. PHZ also causes degradation of spectrin and subsequent lipid peroxidation in the cell membrane skeleton causing cell lysis [18] and changes in cytoskeleton protein cause membrane disintegration [38,39]. Free radical alters the lipid-protein configuration in erythrocyte membrane by different mechanisms i.e. lipid peroxidation, hydrolysis of phospholipid heads, disulfide bridge formation, lipid-lipid crosslink, amino acid residue damage in membrane proteins, lipid-protein crosslink and ultimately reduce the integrity and rigidity of the membrane [40,41]. Hence the changes in mechanical properties of membrane indicate the 2,4-DNPH mediated structural alteration of the cytoskeleton protein. The increase in haemolysis not only indicates the loss of membrane elasticity but also indicates the presence of abnormal flattened cells [28].

In support of the DNPH mediated morphological alterations of RBC and its amelioration by JR extarct, we analyzed the erythrocyte morphology by SEM study, which showed normal biconcave shape with smooth topology of RBC, which was transformed to typical echinocytes due to 2,4-DNPH induction. SEM study also showed that DNPH caused spine-like structures on the surface of the erythrocyte membrane which were mostly corrected in JR extract supplement and the appearance of spine decreased. The roughness of outer surface of RBC membrane was remarkably increased in 2,4-DNPH treatment which was partially prevented by JR supplement. Although, surface roughness of RBC is an independent parameter from the overall cell shape, because only the shape change without changing the roughness indicates no alteration in the membrane cytoskeleton [42]. It was supported by other different studies, where different chemicals and pesticides caused deformation of erythrocyte structure by alteration of membrane lipid [43,44].

Normally, in erythrocyte membrane two leaflets are not separated. But in echinocytes these two leaflets were separated due to uncoupling of hydrophobic interactions caused by free radicals produced in 2,4-DNPH induced anaemia. Again spine in echionocytes are formed possibly by transversal redistribution of membrane phospholipids or due to insertion of amphiphilic compounds into the membrane [45,46]. It has also shown that the conformational changes of integral protein could lead to an expansion of one leaflet of the membrane double layer relative to the other one and in turn results in an alteration in shape. Role of band 3 proteins in these phenomena and the conformation changes have been suggested by Gimsa and Ried in 1995 and Ferrali et al. in 1997 [47,48]. So, erythrocytes of control, JR supplement and JR treated groups showed normal biconcave shape with no such morphological alterations. This might be due to protective nature of JR extract containing various anti-oxidant metabolites i.e. rutin, kaempferol, quercetin etc. Henneberg et al. in 2013 reported that quercetin plays an important role against oxidative damage with preservation of erythrocyte membrane integrity. The presence of catechol in quercetin and B-ring in rutin are essential to sequester free radicals and for antioxidant property [49]. Barreca et al. in 2009 showed that quercetin and rutin have better capacity to sequester free radicals than other flavonoids [50]. Similar observation was also reported earlier by Sen et al. in 2005 [51].

5. CONCLUSION

It can be concluded that the ethanolic extract of *Jussiaea repens* L. can prevent anaemia possibly by stimulating erythropoiesis, Hb synthesis and decreasing haemolysis. It may protect erythrocyte membrane and cytoskeleton disintegration by reducing 2,4-DNPH induced oxidative stress. So, the ethanolic extract of *Jussiaea repens* L. (JR) can be used as a protector of 2,4-dinitrophenylhydrazine (2,4-DNPH) induced anaemia due to its haematinic potential. But further vivid studies are required in dose and duration dependent manner in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. Animal experiments were performed as per the ethical guidelines of Presidency University, Kolkata (Ref. No. PU 796/03/ac/CPSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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