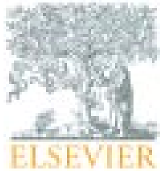

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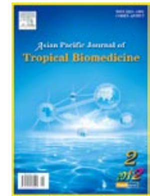

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Screening of herbal extracts influencing hematopoiesis and their chemical genetic effects in embryonic zebrafish

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ABSTRACT

Objective: To screen the herbal extracts influencing the hematopoietic stem cells (HSC) in zebrafish embryos and their chemical genetic effects. **Methods:** The herbals used in this study had been widely applicable in Siddha medicines in South India. Herbal extracts were treated in zebrafish embryos at 4 d post fertilization and the extracts inducing the HSC were enumerated in hemocytometer. The biocompatibility and the organogenesis of the screened extracts were assessed in the zebrafish embryos for their chemical genetic effects. The LC_{50} values were calculated with their parallel control. The blood cells were enumerated. **Results:** The level of RBC was found increased in the *Bergera koenigii* (*B. koenigii*) at 15 μg/mL (P<0.05), *Mimosa pudica* (*M. pudica*) at 20 μg/mL (P<0.05) and *Solanum trilobatum* (*S. trilobatum*) at 25 μg/mL (P<0.05) and decreased RBC level was found in *Phyllanthus niruri* (*P. niruri*) at 30 μg/mL (P<0.05). The WBC count was found increased in *S. trilobatum* at 20 μg/mL (P<0.05) and *Annona muricata* (*Annona muricata*) at 15 μg/mL (P<0.05) and the *Vitis quadrangularis* (*V. quadrangularis*) at 20 μg/mL (P<0.05) decreased the WBC level. There were no notable effects in heart beats and the chemical genetic effects were observed at higher concentration of the extract resulting in Pericardial bulging, trunk tail flexure with heart edema, fin fold deformities *etc.* **Conclusions:** This *in vivo* based screening of Hematopoiesis is an inexpensive assay to screen herbal compounds and found that *S. trilobatum* extract influenced embryonic HSC in zebrafish, which could be a therapeutic for blood related disorders.

1. Introduction

Zebrafish has emerged as an ideal organism for the study of hematopoiesis, by which all types of blood cells are formed from hematopoietic stem cells (HSCs)[1]. Vertebrate hematopoietic cells are self renewing multipotential stem cells during early embryonic development[2]. In zebrafish, Hematopoiesis occurs in a dorsal location above the yolk tube called "the intermediate cell mass" (ICM) which is formed in two paraxial stripes of mesoderm that form during gastrulation[3-5]. Zebrafish embryos are permeable to small molecules making them an ideal model for high-throughput chemical screening of novel bioactive compounds[6]. Hematopoiesis in zebrafish occurs in two waves: the primitive or embryonic wave and the definitive or adult wave. Definitive hematopoietic stem cells arise in the aorta-gonad-mesonephros region AGM[7]. In this study we have concentrated on the influence of herbal small molecules in the adult wave of hematopoiesis. Waves of hematopoiesis occur in a spatially unique manner in zebrafish compared to other vertebrate model organisms. Screening of small molecules represents a powerful tool to study HSCs in zebrafish[7].

Zebrafish developmental hematopoiesis shows close relation to the human and mammalian hematopoietic system and is regulated by conserved molecular pathways[8]. The expression of critical blood genes and the morphology of blood cells are highly conserved in zebrafish and mammals. Hence the changes in zebrafish HSC influencing the herbal small molecules are having a same influence in Humans. Evidence supports that the site of formation of the first definitive HSCs in zebrafish is the AGM (Aorta Gonad Mesonephron Region) which is similar to mammals. These cells subsequently migrate to the kidney, the adult hematopoietic organ in zebrafish, by 5 dpf. The

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intermediate HSC expansion site in zebrafish is the caudal hematopoietic tissue (CHT) which is an analogue of human liver. The hematopoietic progenitors migrate to CHT, located between the caudal artery and veins from AGM[9]. The CHT is a transitional niche to support definitive HSC expansion and maturation in the zebrafish[10]. Hence in our study, the blood cells have been treated at 4 dpf and collected at 5 dpf stage of the zebrafish embryos for the study on HSC changes. Herbal small molecules are considered to be less toxic and induce fewer side effects[11]. Red blood cells (RBC), and white blood cells (WBC) are suspended in the serum in homeostatic concentrations and the influence of HSC are analyzed in this study. The aim of the present study deals with the screening of herbal small molecules influencing the HSC in the embryonic zebrafish. The biocompatibility and chemical genetic effects of the herbal small molecules were determined in the zebrafish by studying the organogenesis effects in the embryos.

2. Materials and methods

2.1. Collection and extraction

Thirty medicinal herbs were collected from Shenbagaramanputhoor, Western Ghats of Kanyakumari, India. All the herbals were washed with tap water and rinsed with distilled water to remove the minerals on the plant materials and shade dried for 25-30 d. The dried herbals were powdered into 1 mm particle size in a mixer grinder. The 10 g of powdered samples were filtered and extracted in Soxhlet apparatus using hexane, chloroform, acetone and methanol on their increasing polarity. After extraction the four different solvent extracts were allowed to evaporate and then concentrated in vacuum concentrator (Eppendorf). The concentrated extracts were collected in 2 mL Eppendorf tubes and stored at 4 ℃[12].

2.2. Breeding and maintenance of zebrafishes

Zebrafishes were bred and maintained in Fish Culture Facility of International Centre for Nanobiotechnology, CMST, M. S. University. Zebrafishes were maintained according to Kannan *et al*[13,14].

2.3. Hematopoiesis-blood cell enumeration

5 μg/mL to 30 μg/mL of 120 herbal extracts (Table 1) were prepared in 1% DMSO as small molecule vehicle in ERS. The herbal extracts were serially diluted and added in the 24 well plate which contained the 4 dpf embryos. The treated and untreated embryos were incubated at 28 ℃ for 12 h. In general the WBC count will be increased during the time of pathogenic infection, hence the embryos were grown in the ERS with anti-infectives (100 μL/L methylene blue and 10 μg/L of streptomycin and nalidixic acid) and the good water quality was maintained. HSCs were isolated and identified from 30 hpf embryos piercing the intermediate cell mass (ICM) region near the yolk and mixed with 5 mL of PBS (pH 7) and observed under the microscope. The WBC and RBC counting of the larval zebrafish embryos were carried out by cutting the tail of the embryos by sterile sharp blade[14] under Microscope (Motic) and 0.5 μL of blood was pipette out using Eppendorf micropipette and diluted in 20 μL (1:40) of WBC dilution fluid (10 mg crystal violet and 1 mL glacial acetic acid in 100 mL with ddH_20) and in 100 μL of (1:200) RBC dilution fluid (860 mg NaCl, 30 mg KCl, 35 mg $CaCl_2$ in 100 mL ddH_2O). A 40 μL was applied and counted in hemocytometer separately for WBC and RBC. The number of cells per cubic millimeter was determined. For WBCs the four fields (top L and R, bottom L and R) indicated squares of blue-stained WBCs were examined under 100× and counted. For RBCs the five fields on the center of hemocytometer (top L and R, bottom L and R, center) were examined in 100× (Motic light microscope) and counted. Cells touching top and right sides were ignored.

2.4. Organogenesis effects of Indian Siddha herbals

Herbal extracts (5-100 μg/mL) influencing the effect of hematopoiesis was treated in the 2 dpf embryos in 48 well microtitre plates. The embryos were incubated at 28 ℃ to observe the organogenesis and chemical genetic effects after 12 h. A parallel control was made during the *in vivo* morphogenesis and chemical genetic studies. The biocompatibility and the physiologic effects of the herbal small molecules were monitored in the developing embryos in the light microscope (Motic) during 2-4 dpf. Rhythmicity/contractility, HBR, blood circulation, intestinal contraction visualization, tail and spinal cord flexure, finfold abnormality, cardiac malformations, yolk sac edema, and eye abnormality were observed for all the extracts influencing the HSC. The organogenesis effects were photographed and analyzed using the ImageJ (NIH) analyzing software.

2.5. Determination of LC_{50}

The LC_{50} analysis was carried out based on OECD Guideline for Fish Embryo Toxicity (FET) test[15,16]. The embryos were statically exposed (10 embryos/well) to the crude plant extracts in 24-well multi-plates with ten concentrations (10-100 μg/mL) and a control group with triplicates. Test solutions of the selected concentrations were prepared by dilution of a stock solution. The stock solutions were prepared by simply agitating the crude extracts with 1% DMSO in the embryo rearing solution by Vortex mixture (Genei). The eggs were carefully handled to minimize any stress to the embryos. Healthy embryos from 24 hpf were separated using cut tips under 10× magnifications in light microscope (Motic) and transferred into the test solutions. It was maintained and observed for 24, 48, 72 and 96 h periods after treatment. The calculation was carried out for 96 h. For every 24 h the embryo rearing solutions were replaced. For calculation of the LC_{50} value and the confidence limits (95%) statistical methods were applied[17].

2.6. Statistical analysis

Each experiment was performed at least three times, and all values presented are the means±SD of triplicate assays.

T-test was used to analyze the statistical significance of the results. *P* values $<$ 0.05 were considered statistically significant.

3. Results

3.1. Hematopoiesis-identification of blood cells

The progenitor embryonic hematopoietic cells were identified from the 30 hpf embryos in the ICM region near the dorsal yolk sac region and shown in Figure 1a. Hematopoietic stem cells of definitive waves were identified at the 4 dpf stages of the zebrafish embryos by giemsa staining (Figure 1b and c) based on their cell morphology[18]. The hematopoiesis waves (Primitive and Definitive) of zebrafish ontogeny were represented in Figure 1d with the permission of authors Jing and Zon[19]. The cells were identified as erythrocytes, thrombocytes, eosinophils, neutrophils, monocytes and lymphocytes as shown in Figure 2. The blood cells were identified based on the methods of Claver and Quaglia[18].

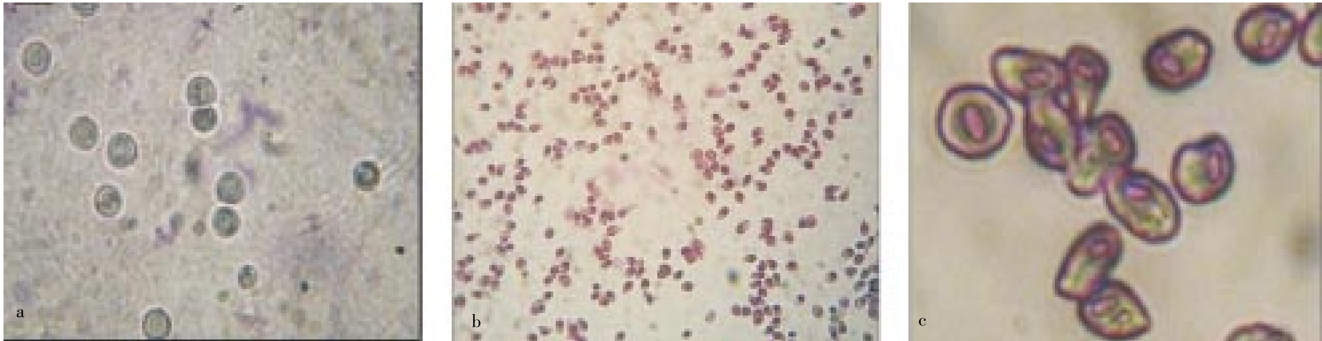

Figure 1

a) Giemsa staining of 30 hpf progenitor cells (100×). b) Blood cells at 40X magnification (Giemsa staining) 4dpf. c) Blood cells at 100× magnification (Giemsa staining) 4 dpf.

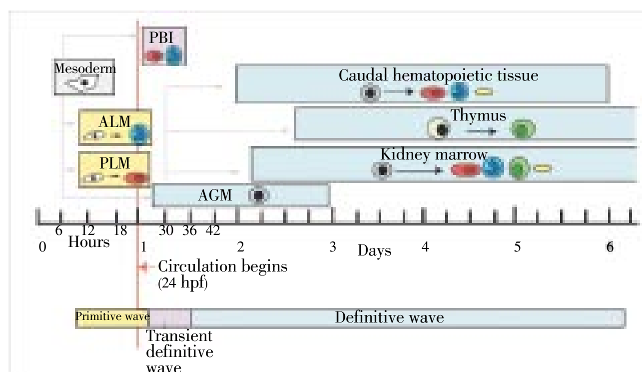

Figure 1.

d) Ontogeny of zebrafish showing primitive wave and definitive wave of hematopoiesis[19] with the permission of authors from Harvard Medical School, Boston, USA.

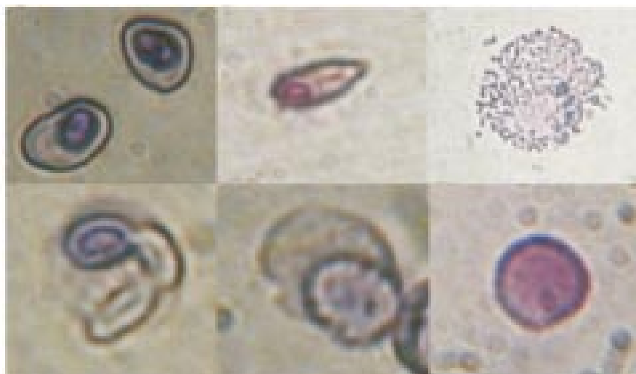

Figure 2

Identification of the hematopoietic stem cells (Blood cells) in zebrafish embryo at 4 dpf. a) Erythrocyte, b) Thrombocyte, c) Eosinophil, d) Neutrophil, e) Monocyte, f) Lymphocyte (T/B cell).

3.2. Increase of RBC

Methanolic extract (15 μg/mL) of *Bergera koenigii* (*B. koenigii*)induces the RBC level at 5 dpf zebrafish embryos. A 51% of increased in RBC count was identified. The RBC count was increased and resulted in $(0.373\,3\pm0.005\,7)\times10^6/mm^3$ and the control showed $(0.246\,7\pm0.015\,2)\times10^6/mm^3$ with the *P* value of 0.000 088 (Figure 3). Methanol extract of *Mimosa pudica* (*M. pudica*) induced the RBC level at 20 μg/mL and showed a range of $(0.370\,0\pm0.017\,3)\times10^6/mm^3$ and the control had $(0.273\,3\pm0.020\,8)\times10^6/mm^3$ and is shown in Figure 4 with the *P* value of 0.001 739. A 35% of increased in RBC count was identified. Hexane extract of *Solanum trilobatum* (*S. trilobatum*) induces the increase in RBC level at 25 μg/mL showed $(0.376\,6\pm0.005\,7)\times10^6/mm^3$ and the control had $(0.246\,7\pm0.011\,5)\times10^6/mm^3$ with the *P* value of 0.000 03 (Figure 5). A 53% of increased in RBC count was identified.

3.3. Decrease of RBC

Treatment of acetone extracts of (30 μg/mL) *Phyllanthus niruri* (*P. niruri*) showed decrease in RBC level as $(0.163\,3\pm0.011\,5)\times10^6/mm^3$ and in control showed $(0.293\,3\pm0.015\,2)\times10^6/mm^3$ with the *P* value of 0.000 15 (Figure 6). A 44% of decreased in RBC count was identified.

3.4. Increase of WBC

Hexane extract (20 μg/mL and 30 μg/mL) of *S. trilobatum* showed the increase in the WBC level $(37.666\,7\pm0.577\,4)\times10^2/mm^3$ and $(38.666\,7\pm0.577\,4)\times10^2/mm^3$ respectively but the

control had (24.666 7±0.577 4)×10^2/mm^3) in the 5 dpf embryos were depicted in Figure 7 with the P value of 0.000 005. A 53% of increased in WBC count was identified. Similarly, Acetone extract (15 μg/mL) of *Annona muricata* (*A. muricata*) increased WBC level showed (34.666 7±1.527 5)×10^2/mm^3 and control results were (30±1)×10^2/mm^3 and were shown in Figure 8 with the P value of 0.005 723. A 16% of increased in WBC count was identified.

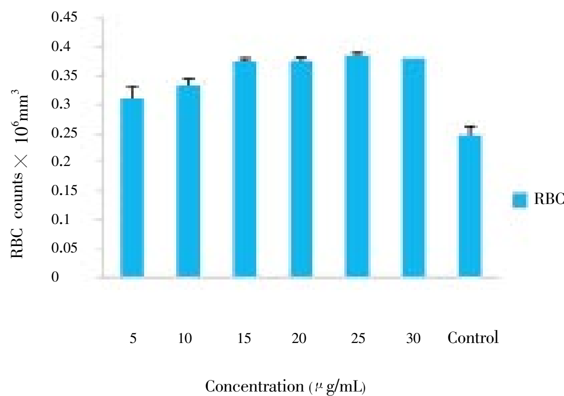

Figure 3
Increase of RBC count in embryonic zebrafish by methanolic extract of *B. koenigii*.

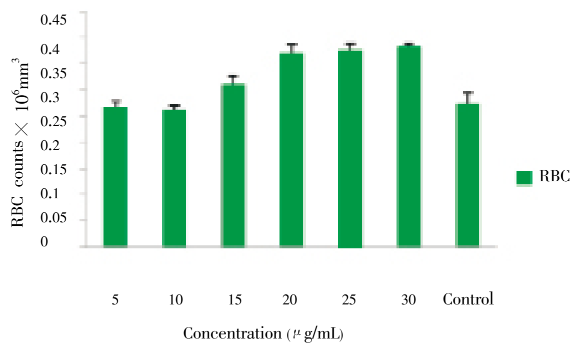

Figure 4
Increase of RBC count in embryonic zebrafish by methanolic extract of *M. pudica*.

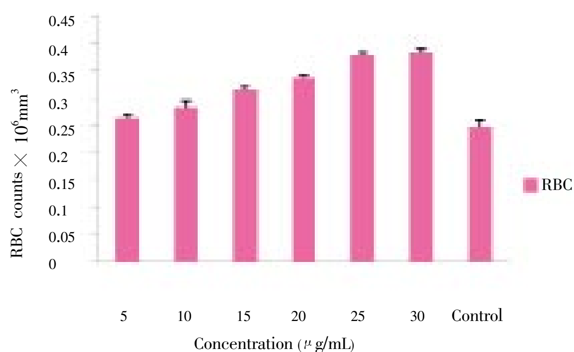

Figure 5
Increase of RBC count in embryonic zebrafish by hexane extract of *S. trilobatum*.

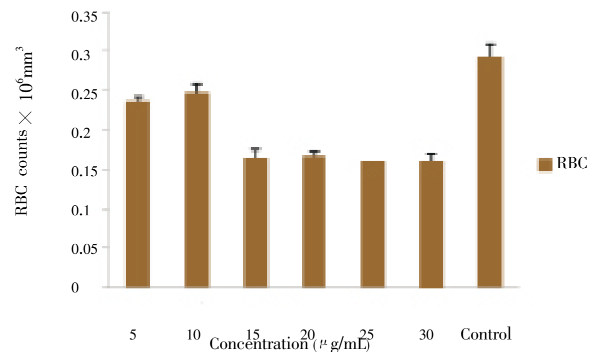

Figure 6
Decrease of RBC count in embryonic zebrafish by acetone extract of *P. niruri*.

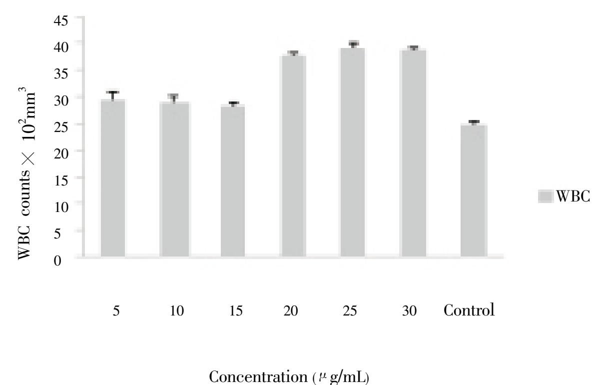

Figure 7
Increase of WBC count in embryonic zebrafish by hexane extract of *S. trilobatum*.

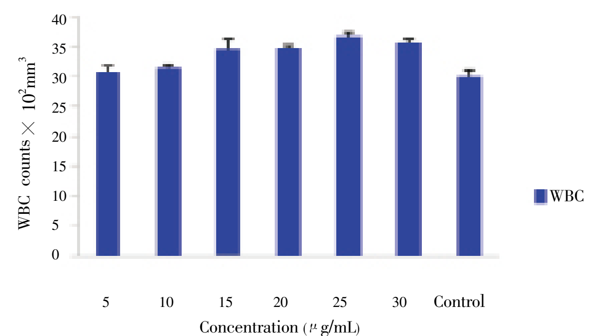

Figure 8
Increase of WBC count in embryonic zebrafish by acetone extract of *A. muricata*.

3.5. Decrease of WBC

Acetone extract (20 μg/mL) of the *V. quadrangularis* inhibited the WBC level (20.666 7±0.577 4)×10^2/mm^3 and in control the results were (33.333 3±2.081 6)×10^2/mm^3) in the

5 dpf embryos with the *P* value of 0.000 265 and shown in Figure 9. A 38% of decreased in WBC count was identified.

3.6. Enumeration and quantification of blood cells

The quantification of RBC and WBC level was calculated from the obtained results of blood cell counting. *S. trilobatum* showed maximum stimulation of RBC in the embryonic hematopoietic stem cells when compared to *B. koenigii* and *M. pudica* and are shown in Figure 4, 5 and 6 after 24 h incubation. *P. niruri* was the only small molecule source which reduces the RBC count. *S. trilobatum* showed 52.7% of increasing WBC level when compared to *A. muricata*, which showed 15.6% increase. *V. quadrangularis* showed a 38% of decreases in the WBC of 5 dpf zebrafish. The percentage of increase and decrease of RBC and WBC were analyzed and is shown in Figure 10 and 11. All the results were analyzed with *t*-test with *P* values <0.05 were considered statistically significant.

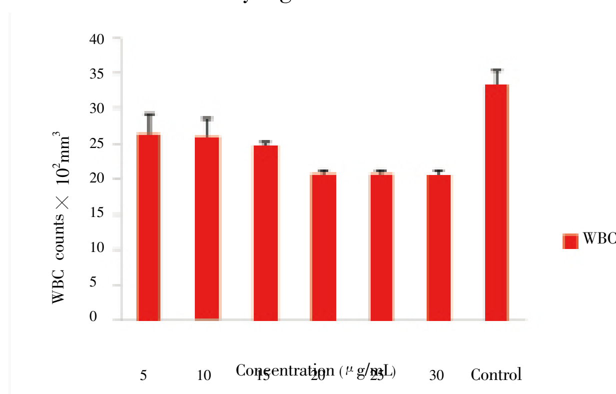

Figure 9

Decrease of WBC count embryonic zebrafish by in acetone extract of *V. quadrangularis.*

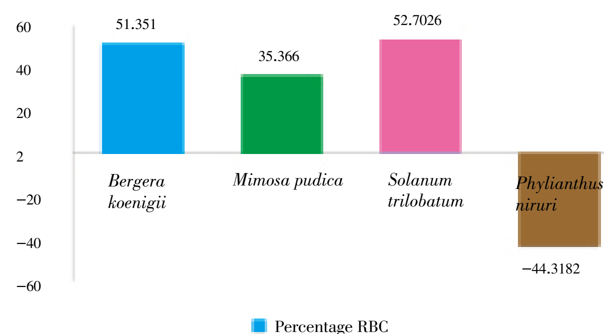

Figure 10

Percentage of RBC level influenced by herbal small molecules from *B. keonigii, M. pudica, S. trilobatum* and *P. niruri* treated at 4 dpf in zebrafish with their controls.

3.7. Physiological effects of herbal small molecules

The herbal small molecules influencing HSCs did not show any change in heart beat rate (HBR) and is shown in Table 1. The organogenestic studies showed that the small molecules induce the genetic effects of the embryos in higher concentrations. The cardiac malformations were observed in all the HSC inducing extracts at 3 dpf - 5 dpf at its LC_{50} concentrations. Pericardial bulging and yolk sac edema were observed in *B. koenigii*. Details of the deformities and chemical genetic effects during organogenesis were explained for *M. pudica, S. trilobatum, P. niruri, A. muricata* and *V. quadrangularis* and each are shown in Figure 12a-g. *V. quadrangularis* showed tail and trunk flexure, truncation in the spinal cord was observed (Figure 12f).

Small molecule from *S. trilobatum* showed lesser toxicity in its LC_{50} concentrations and *A. muricata* extract showed inconsistent blood flow, some of the embryos showed deformity in the trunk and gut region. Flow rate was highly reduced when compared to the control. Blocks were observed in the blood vessels and the blood cell movement was not seen in caudal and trunk region of zebrafish in higher concentration.

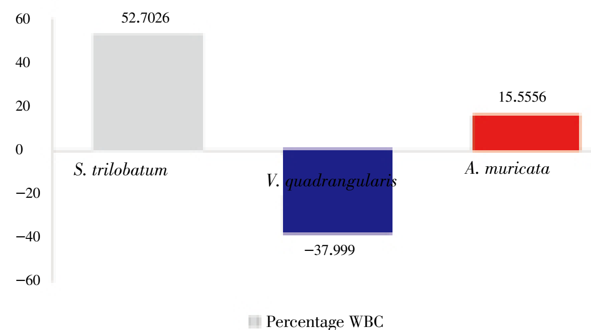

Figure 11

Percentage of WBC level influenced by herbal small molecules from *S. trilobatum, V. quadrangularis* and *A. muricata* treated at 4 dpf in zebrafish with their controls.

Table 1

Determination of HBR in zebrafish embryos at 4 dpf after 6 h treatment.

Herbal extract	HBR/15 sec
Control	41.00±1.00
B. koenigii – methanol	40.00±0.00
M. pudica – methanol	41.00±2.00
S. trilobatum – hexane	39.67±2.08
P. niruri – acetone	40.33±2.08
V. quadrangularis – acetone	39.33±1.15
A. muricata – acetone	41.00±3.46

Table 2

Determination of LC_{50} values for the herbal extracts influencing hematopoiesis in zebrafish embryos.

Herbal extract	LC_{50} value (μg/mL)	95% Confidence limits	
		Lower (μg/mL)	Upper (μg/mL)
A. muricata – acetone	60.49	54.51	66.08
B. koenigii - methanol	47.80	41.79	53.25
S. trilobatum– hexane	45.78	39.03	51.92
M. pudica – methanol	50.17	44.17	55.64
P. niruri - acetone	57.43	50.65	63.85
V. quadrangularis - acetone	41.14	34.69	46.95

3.8. Determination of LC_{50}

The LC_{50} value was calculated based on their OECD regulation. The LC_{50} value was calculated by probit analysis and tabulated in Table 2.

Figure 12

a. Methanolic extract of *B. koenigii* (45–55 μg/mL) treated embryos (3 dpf). i) Pericardial bulging with yolk sac edema. Scalebar 200 μm. ii) Pericardial bulging with malfunction of heart edema dorsal trunk region and fin fold abnormality. Scalebar 150 μm. iii) Bradycardia with pericardial bulging. Scalebar 200 μm.
b. Methanolic extract of *M. pudica* (45–55 μg/mL) treated embryos (3 dpf). i) Trunk flexure. Scalebar 250 μm. ii) Aggregation of HSC. Scalebar 50 μm.iii) Pericardial bulging with finfold deformity 200 μm
c. Hexane extract of *S. trilobatum* (40–50 μg/mL) treated embryos (3 dpf). i, ii) Mild Pericardial bulging. i) Scalebar 250 μm. ii) Scalebar 50 μm. iii) Fin flexure in the Caudal region. Scalebar 250 μm.
d. Hexane extract of *P. niruri* (50–60 μg/mL) treated embryos (3 dpf). i) Affected the entire muscle tissue development in the organogenesis. Pericardial bulging, head edema with protruded eyes and trunk flexure. Scalebar 150 μm. ii) Pericardial bulging. Scalebar 150 μm. iii) Cardiac deformities with tail flexure. Scalebar 200 μm.

Figure 12

e. Acetone extract of *A. muricata* (55–65 μg/mL) treated embryos i) Pericardial bulging. Scalebar 50 μm (3 dpf). ii and iii) Trunk flexure. Scalebar 100 μm. (3 dpf).
f. Acetone extract of *V. quadrangularis* (35–45 μg/mL) treated embryos (3 dpf). i) Pericardial bulging with yolk sac edema, head edema and trunk flexure. Scalebar 150 μm. ii) Aggregation of hematopoietic blood cells in the yolk sac region. Scalebar 150 μm. iii) Yolk sac edema and trunk flexure. Scalebar 150 μm.
g. Control embryos. i) 2 dpf. ii) 3 dpf. iii) 4 dpf (Days post fertilization).

Table 1

Name of plants for herbal extracts.

Sl. No.	Name of plants
1	*Acalypha indica*
2	*Achyranthus aspera*
3	*Adathoda vasica*
4	*Alternanthera sessilis*
5	*Amaranthus viridis*
6	*Annona muricata*
7	*Annona squamosa*
8	*Bergera koenigii*
9	*Cassia fistula*
10	*Cardiospermum halicacabum*
11	*Crotalaria verucosum*
12	*Cynodon dactylon*
13	*Datura alba*
14	*Eclipta alba*
15	*Emblica officinalis*
16	*Ervatamia coronaria*
17	*Ixora coccinae*
18	*Leucas aspera*
19	*Mimosa pudica*
20	*Moringa oleifera*
21	*Pedalium murex*
22	*Pergularia daemia*
23	*Phyllanthus niruri*
24	*Sida acuta*
25	*Solanum nigrum*
26	*Solanum trilobatum*
27	*Solanum xanthocarpum*
28	*Tephrosia purpurea*
29	*Tridax procumbens*
30	*Vitis quadrangularis*

4. Discussion

Chemical screens from herbal small molecules have the potential to identify lead compounds for novel therapeutics for blood related diseases. In the present study, the analysis was done in the induction of HSC in the embryonic stages of zebrafish. It was screened 120 organic extracts from 30 herbals which are commonly used in traditional medicine in South India and found that herbal extracts influenced the hematopoiesis. The HSCs were isolated from the caudal region of the zebrafish embryos for blood cell enumerations. Methanolic extract of *B. koenigii*, *M. pudica* and hexane extract of *S. trilobatum* induces the RBC count. These small molecules might be inducing the regulation of progenitor cell of the HSC or any regulatory factor for the stimulation of erythrocyte production. Similar studies were carried out by North *et al*[20] by identifying the new regulators of HSC from the chemical screens which increases the HSC number. Small molecules from acetone extract of *P. niruri* reduced the RBC count in the zebrafish embryos. Another studies by Yeh *et al*[21] screened the small molecule that suppress

the inducible erythropoiesis to granulopoiesis conversion. However the herbal small molecule proved that they have the potential of influencing the erythropoiesis and influence HSC production. Evidence in the present study stimulating the embryonic erythropoiesis by the report on *G. conruana* seed extract might be effective through an influence on the stimulant cytokine erythropoietin[22]. Zebrafish has many appealing features, such as the ease of manipulation of transparent embryos and the capacity to carry out large-scale and chemical screens[23]. At 24 hpf, these primitive blood cells start to circulate throughout the embryo. Subsequently, the definitive HSCs emerge from the ventral wall of the dorsal aorta[24,25], and these HSCs migrate to the posterior region in the tail called the caudal hematopoietic tissue (CHT)[9,10] and in this study an identification was done for HSC at 30 hpf. Previous research has shown that prophylactic and therapeutic oral administration of anti-oxidant supplement significantly increased cells of hematopoietic origin in animals exposed to potentially lethal dose[26].

The direct link between anti-oxidant activity and hematopoietic boosting effect was also demonstrated by the observation that ascorbic acid supplementation, through its action as a free radical scavenger, increased significantly the hemoglobin levels of children suffering from sickle cell anemia[27]. Two teams of researchers at different times and locations have also demonstrated increases in RBC parameters on the effect of the *Garcinia conruana* seed extract on rats and rabbits respectively[22,28,29]. Similarly, in this study it was observed the increase of embryonic erythrocytes in the HSC *in vivo* in zebrafish treated with methanol extract of *B. koenigii*, *M. pudica* and hexane extract of *S. trilobatum*. This observation suggested that to increase in embryonic RBC may be effected through an influence on the stimulant cytokine erythropoietin[30,22]. Hexane extract of *S. trilobatum* and acetone extract of *Annona muricata* induces the WBC count in the zebrafish embryos. Increases in lymphocytes count are observed in *G. kola* extract [31]. However, the acetone extract of *V. quadrangularis* reduces WBC count in the zebrafish embryos at 5 dpf. In the present study it was confirmed that the hexane extract of *S. trilobatum* had highly influenced the hematopoiesis process in zebrafish and this is supported by the research works which also show the increase of RBC and WBC in *S. macrocarpum*[32]. During 4 dpf, HSCs seed the kidney marrow, which is equivalent to bone marrow in mammals. Based on the above information on the formation of HSC, the small molecules were treated at 4 dpf, since it is a definitive stage of hematopoiesis which gives rise to HSCs that generate the full range of blood cell types in the later embryo and throughout adulthood[33,34]. In the present study, the caudal region of the tail was cut[13,14] for the collection of blood cells for enumeration study.

Zebrafish and other vertebrate animals share similar genetic programs that regulate hematopoiesis. Hematopoiesis programs are largely conserved between mammals and zebrafish[35], and the genetic amenity of the zebrafish has advanced its cause as a strong model system for developmental studies[36]. Hypothetically the herbs influencing the HSC could be stimulating the progenitor cell mechanism/regulation for the production or suppression of HSC. The quantification of the WBC and RBC in the embryonic fish is a first (*in vivo*) study and is significance showed that the herbal small molecules were highly influenced the HSC counts. The study[37,32] proved *Solanum* sp. stimulated RBC and it was identified in the present study that the hexane extract of *S. trilobatum* stimulate the embryonic hematopoiesis. The RBC and WBC level is increased at a percentage of 58.7% and 52.7% respectively. They showed statistically significant P value of $P < 0.05$ in relation to control. Intra embryonic blood cell formation and the migration of these cells into the yolk sac was confirmed by live imaging in zebrafish[38] and it was observed the migration of cells into the yolk from 3 dpf to 5 dpf embryos with light microscope (Motic). An aqueous extract of *Rehmannia glutinosa* exhibited a significant angiogenesis effect with an increase in capillary sprout formation in subintestinal vessel of zebrafish embryos[39]. The organogenesis and chemical genetic effects on the zebrafish embryos were studied through the small molecule that influences the hematopoiesis in zebrafish.

We presume that the small molecules in ERS passively diffuse into developing embryos via chorion pore channels which creates specific organogenesis and phenotype effects in a dose dependent manner. Studies on the chemical genetic effects are highly influenced by the small molecules in high concentrations. This *in vivo* based screening of small molecules would be a simple approach for the screening of drugs for blood related diseases. Our result showed that herbal molecules induce the production of RBC and WBC, which could be a therapeutic lead for anaemia/AIDS/leukemia and other several blood related diseases.

In conclusion this *in vivo* based screening of small molecules would be a simple approach for the screening of drugs for blood related diseases. This result showed that molecules are proved to influence the production of embryonic HSC, which could be a therapeutic for Leukemia and other blood related diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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