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Exploration of the Hematological Profile of Male Sprague-Dawley Rats after Chronic Administration of an Ayurvedic Formulation Punarnavasava (PRV) Used in Ascites Treatment

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Abstract

Punarnavasava (PRV) is a classical Ayurvedic preparation and is very popular in rural areas due to its usage in the treatment of ascites. This study was conducted to explore the hemotoxicological effect of PRV after chronic administrations for 45 days to male Sprague-Dawley rats. After the observations, there were statistically significant changes in Basophil and Lymphocyte count (p=0.020 and p=0.032 respectively) but showed negligible changes for other hematological parameters like RBC, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Erythrocyte sedimentation rate (ESR), etc. From these results, it can be concluded that PRV is less hemotoxic at higher doses and thus can be recommended to use safely in the treatment of ascites.

Keywords: Punarnavasava; Ascites; Hemotoxicity; Ayurvedic; RBC; WBC

Abbreviations: HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; ESR: Erythrocyte Sedimentation Rate; BT: Bleeding Time; CT: Clotting Time; MPV: Mean Platelet Volume; PCT: Plateletcrit; PDW: Platelet Distribution Width.

Introduction

For several hundred decades, the application of ayurvedic medicine has been a part of the treatment processes in the Indian subcontinent. It is considered as one of the oldest and safest sources of medication [1,2]. World Health Organization (WHO) stated that approximately 70%-80% of populations of the world depend on nonconventional therapies that primarily come from natural sources [3]. This wide range of Ayurvedic medicine is due to the huge natural sources, easy accessibility, easy affordability, and healing potential that are also proved by numerous investigations [4]. For the treatment of different ailments in a promising manner, the use of herbal medicines is a great source in Bangladesh and other South Asian countries. Ayurvedic medicine is working as a science of life with a holistic approach to the development of personalized medicine as well as the development of public health. Furthermore, Ayurvedic medicine is a plenary healthcare system that comprises the physical, psychological, and spiritual health of the people [5-7]. The use of Ayurvedic medicine is considered as a part of primary health care facilities in this subcontinent [1,2]. Traditional medicines have contributed to the development of numerous novel therapeutic drugs when compared to other drug sources for the prevention of diseases [8].

The term ascites is used to define as a gastroenterological disorder that leads to the accumulation of fluids in the peritoneal cavity and that may exceed up to 25 ml [9]. Ascites is one of the most prevalent complications of liver cirrhosis that accumulates due to portal hypertensive disorder. Approximately 15% of cases of ascites are caused due to non-hepatic fluid retention in the liver. More than 50% of patients with liver cirrhosis may develop ascites in 10 years following their diagnosis [10]. After the development of ascites, the condition of the patient worsens with a 1-year mortality rate of approximately 15% and a 5-year mortality rate of approximately 44% [11].

Punarnavasava (PRV) or Punarnavasavam is a liquid Ayurvedic formulation that is prepared by the fermentation process of some valuable medicinal herbs that have a beneficial effect on the liver, spleen, heart, kidney, and stomach. The formulation of Punarnavasava (PRV) is stated in Table 1 [12,13]. Punarnavasava (PRV) can be used in the management and treatment of spleen disorder, ascites, abdominal disease, edema, and inflammation stated in ancient Bhaishajya Ratnavali. Currently, this preparation is very popularly used in the treatment of ascites [14]. Bangladesh National Formulary of Ayurvedic Medicine included Punarnavasava (PRV) on page no 145 in 1992 [15]. Permission to manufacture at the industrial scale is printed on page no. 535 (column 1: Product code 16.41). Directorate of Drug Administration has issued a license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published Bangladesh Gazette 24 Part VI dated Thursday, June 11th, 1998). In many cases, the application of Ayurvedic medicine helps to avoid expensive and extensive procedures of clinical investigations. It is a matter of regret that Ayurvedic medicines are most often neglected for the investigations of hemotoxicological studies. However, at present, a good number of Ayurvedic manufacturers are formulating and marketing the Classical

Ayurvedic Medicinal Preparation. Keeping in mind the present scenario, Ayurvedic preparation of Punarnavasava (PRV) has been accomplished to discover a wide spectrum of its hematological parameters by utilizing experimental animals. The objectives of this study are to have a better understanding of the possible hemogram profile of PRV and to justify the safety of users of this drug in the treatment of ascites.

S.No	Components	Scientific Name	Amounts
1	Sunthi (Rz.)	*Zingiber officinale Roxb.*	48 g
2	Marica (Fr.)	*Piper nigrum Linn.*	48 g
3	Pippali (Fr.)	*Piper longum Linn.*	48 g
4	Haritaki (Fr. P.)	*Terminalia chebula Retz*	48 g
5	Bibhitaka (Fr. P)	*Terminalia belericaRoxb*	48 g
6	Amalaki (Fr. P.)	*Tamarindus indica Linn.*	48 g
7	Darvi (daru haridra) (St.)	*Berberis vulgaris*	48 g
8	Svadamstra (goksura) (Fr.)	*Tribulus terrestris Linn.*	48 g
9	Brhati (Rt.)	*Solanum indicum Linn*	48 g
10	Kantakari (Pl.)	*Garcinia morella*	48 g
11	Vasa (mula) (Rt.)	*Adhatoda vasaka Nees*	48 g
12	Eranda mula (Rt.)	*Ricinus communis Linn*	48 g
13	Katuki (Rt.)	*Luffa Amara Roxb.*	48 g
14	Gajapippali (Fr.)	*Scindapsus officinalis Schott*	48 g
15	Sothaghni (punarnava) (Rt.)	*Boerhaavia diffusa Linn.*	48 g
16	Picumarda (nimba) (St. Bk.)	*Azidiracta indica A Juss.*	48 g
17	Guduci (St.)	*Tinospora cordifolia Miers*	48 g
18	Suska (mulaka) (Rt	*Raphanus sativus Linn*	48 g
19	Duralabha (Rt.)	*Fagonia cretica Linn*	48 g
20	Patola (Lf.)	*Trichosanthes dioica/lobata Roxb*	48 g
21	Dhataki (Fl.)	*Woodfordia fruticosa*	768 g
22	Draksa (Dr. Fr.)	*Vitis vinifera Linn.*	960 g.
23	Sita (sarkara)	*Pseudarthia viscida*	4.800 kg.
24	Maksika (madhu)	*Madhuca indica*	2.400 kg.
25	Water		24.576 L.

Table 1: Name of ingredients used in the formulation of ayurvedic preparation of punarnavasava (PRV).

Methods

Collection of punarnavasava (PRV) and other reagents

Punarnavasava (PRV) plants were collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh for this research work. Ketamine injection used for anesthesia was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other chemicals and reagents used in this study were purchased from Human GmbH, Wiesbaden, Germany.

Experimental animals

For this toxicological research work, healthy Albino rats (Rattusnovergicus: Sprague-Dawley strain) of eight-week-old of both sexes were used. These animals were weighed about 50-70 g. The rats were maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University. Before the experiment, Rats were randomly divided into 4 groups of 10 animals/sex. Thus, ten rats were taken for each group for both the control and the experimental group. All of the rats were kept in plastic cages having dimensions of (30 × 20 ×13) cm and softwood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water and maintained at the natural day-night cycle. The animals were housed in a well-ventilated hygienic experimental animal house. Constant environmental parameters with adequate nutritional conditions were maintained. The rats were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guidelines for the care and use of laboratory animals. The experimental animals were marked carefully on the tail which helped to identify a particular animal.

Design of the study

Acute toxicity study: The acute oral toxicity test was carried out following the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing of chemicals and, the drug with minor modifications [16]. Sixteen female mice (non-pregnant, 30-35 g body weight) were divided into four groups of four animals each. Different doses of experimental drug (PRV) (50 ml/kg, 60 ml/kg, 70 ml/kg, and 80 ml/kg) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following PRV administration.

Chronic toxicity studies: Before starting the experiment, Sprague-Dawley rats were randomly divided into 2 groups, each with 8 animals. One group was treated with PRV and another was used as a control group. The control animals were administered with distilled water only as per the same volume as the drug-treated group for 45 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 40 ml/Kg body weight [17]. After aclimatization, PRV preparation was administered to the rats by an intra-gastric syringe between the 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guidelines for the care and use of laboratory animals. The experimental animals were marked carefully on the tail which helped to identify a particular animal. By using an identification mark, responses were noted separately for a particular period before and after the administration [18].

Blood samples collection

At the end of the 45-day treatment period, the animals were fasted for 18 hours and also 24 hours after the last administration. Ketamine (500 mg/kg i.p.) was administered for anesthesia. Whole blood samples were collected from post vena cava and transferred to EDTA-added tubes immediately. All analyses were completed within 12 h of sample collection [19].

Determination of hematologic parameter

Studies of hematologic profile involve analysis of parameters such as WIC (WBC Impedance Count) and Red Blood Cells (RBCs) level was determined by Electrical Impedance method [20]. Hemoglobin (HGB) level was determined by Modified hemoglobin cyanide method [21]. Platelet level was determined by Electrical Impedance method [20]. WOC (WBC Optical Count) level determined by Laser light scatter [22]. Differential Analysis was done by the CELL-DYN 3700 System. Erythrocyte Sedimentation Rate is determined by Wintergreen Method [23]. For the determination of the bleeding time, the modified procedure of Dejana et al. was used [24]. Clotting time was determined with the method of Goldstein [25]. MCV, MCH, MCHC, HCT, and PCT was calculated according to the formula as given by Ketley and Diem [26,27].

$MCV=[HCT(\%)/RBC\ count\ (millions)] \times 10$

$MCH=(HGB/RBC) \times 10$

$MCHC=[Hb\ (g/dL)/HCT(\%)] \times 100$

$HCT=(RBC \times MCV)/10$

$PCT=(PLT \times MPV)/10,000$

Differential count of Neutrophil (%)=Number of Neutrophil/Total number of Leucocytes

Differential count of Eosinophil(%)=Number of Eosinophil/Total number of Leucocytes

Differential count of Basophil(%)=Number of Basophil/Total number of Leucocytes

Differential count of Lymphocytes(%)=Number of Lymphocytes/ Total number of Leucocytes

Differential count of Monocytes(%)=Number of Monocytes/Total number of Leucocytes

The platelet distribution width (PDW) is a measure of the heterogeneity of the PLT population and the Red cell distribution width (RDW) is a measure of the heterogeneity of the RBC population.

Statistical analysis

The group data are expressed as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 22) was applied for the analysis of data. Differences between groups were considered significant at $p<0.05$, 0.01, and 0.001.

Results

Acute toxicity study results

No mortality was found when the drug (PRV) administered up to a high dose of 80 ml/kg. Thus, the LD50 value was found to be greater than 80 ml/kg body weight. Besides, the animals did not manifest any signs of respiratory distress, restlessness, general irritation, or convulsion. Since PRV is used for the clinical purpose of treatment of liver disease, diabetes, bacteria, virus, diuresis, immunosuppression, inflammation cardiac disease, and cancer for many years, a limit test was performed in acute oral toxicity study. As per OECD test guideline 425, when there is enough information to support that a specific material show immortality and non-toxic nature then limit test at highest starting dose (80 ml/kg body weight) then conducted. No signs of toxicity and mortality observed at dose 80 ml/kg body weight. Therefore, it can be concluded that PRV when administered at a single dose is non-toxic and can be used safely in oral formulations.

Chronic hematologic profile study results

In this experiment, the Total Count (TC), Differential Count (DC), various erythrocyte parameters, platelet parameters, ESR and blood bleeding time and clotting times of Sprague-Dawleyrat were determined after 45 days chronic administration of Punarnavasava (PRV).

Effect of punarnavasava on different blood cell and hemoglobin count in male rats

Comparing with the control group a statistically insignificant and negligible increase (3.36%, p=0.76) observed in the number of white blood cell count and statistically noticeable decrease (8.03%, p=0.076) of the red cell of male rat observed. Again, a statistically insignificant (9.74%, p=0.666) increase in the number of platelet count noticed. Statistically prominent and high increase observed in both Neutrophils and Eosinophils count (37.17%, p=0.105 and 150.0%, p=0.32). On the other hand, statistically insignificant and negligible decreases observed in both Lymphocytes and Monocytes (6.17%, p=0.53 and 1.08%, p=0.96). All of those results are presented in Table 2.

Parameters and Units	Control (Mean ± SEM)	PRV (Mean ± SEM)	% Change
WBC (thousand/µl)	5.302 ± 0.3984	5.480 ± 0.4160	Increase 3.36
RBC (million/µl)	7.120 ± 0.0880	6.548 ± 0.2660	Decrease 8.03
Platelet(thousand/µl)	461.800 ± 7.2484	506.800 ± 96.5936	Increase 9.74
Neutrophil (thousand/µl)	1.060 ± .0772	1.454 ± 0.2016	Increase 37.17
Eosinophil (thousand/µl)	0.012 ± 0.0120	0.030 ± 0.0122	Increase 150
Basophil (thousand/µl)	0	0	-

Lymphocyte (thousand/µl)	4.004 ± 0.3255	3.754 ± 0.2010	Decrease 6.17
Monocyte (thousand/µl)	0.186 ± 0.0081	0.184 ± 0.0484	Decrease 1.08

Table 2: Effect of Punarnavasava (PRV) on different blood cell count.

Effect on differential WBC count in male rats

Blood cell count result obtained by the Electrical Impedance method on blood sample of 45 days Punarnavasava administered rat shows an insignificant but prominent increase (21.75%, p=0.101) in the percentage of the Neutrophil count. There is a statistically insignificant (16.63%, p=0.750) decrease in the percentage of the Eosinophil count of the male rat observed. But a statistically significant (41.54%, p=0.020) increase in the percentage of Basophil count of the male rat noticed. On the other hand, statistically significant decrease (8.34%, p=0.032) in the percentage of Lymphocyte count observed. There is a statistically prominent and very high increase (181.52%, p=0.148) observed in the percentage of Monocyte count of the male rat. All the findings are summarized in Table 3.

Parameters (%)	Control (Mean ± SEM)	PRV (Mean ± SEM)	% Change
Neutrophil	20.780 ± 0.2709	25.300 ± 2.1272	Increase 21.75
Eosinophil	0.0878 ± 0.03359	0.0732 ± 0.02893	Decrease 16.63
Basophil	2.080 ± 0.0520	2.944 ± 0.2358	Increase 41.54
Lymphocyte	76.520 ± 0.4152	70.140 ± 2.0098	Decrease 8.34
Monocyte	0.5444 ± 0.15854	1.5326 ± 0.54776	Increase 181.52

Table 3: Effect punarnavasava (PRV) on differential (WBC) count in male rats.

Effect of punarnavasava on hemoglobin, HCT, MCV, MCH, MCHC, RDW, ESR, BT, CT, MPV, PCT, and PDW in male rats

After 45 days of long administration of Punaravasa (PRV) at a dose of 40 ml/kg on male rat, the blood sample analysis reports recommend that the Hemoglobin content of the blood decrease (6.3%, p=0.264) which is statistically not significant but prominent. At the same time, HCT level of the blood also decreased noticeably (9.4%,p=0.058). The red cell index of male rat Mean corpuscular volume (MCV) also decreased slightly (1.46%,p=0.18). A slight decrease (1.37%) observed in another red cell index Mean corpuscular hemoglobin (MCH). There is a negligible and statistically insignificant decrease (0.14%, p=0.95) observed in the Mean corpuscular hemoglobin concentration. A statistically noticeable increase (4.65%,p=0.065) found in another red cell indexred cell volume distribution width (RDW).There is a statistically insignificant(8.33%,p=0.58) increase noticed in the Erythrocyte sedimentation rate (EDR) in blood from the male rat. Remarkable shortening (12.5%, p=0.195) of rat cutaneous tail bleeding time observed and the result is statistically prominent. The whole blood clotting time (CT) also shortened (1.37%,p=0.68)slightly in the male rat.

There is a statistically insignificant increase in both the mean platelet volume (MPV) and Platecrit value (PCT) of the male rat(1.36%,p=0.58) and (9.76%,p=0.64) noticed on the other hand platelet volume distribution width (PDW) decrease insignificantly (0.28%,p=0.89)after 45 days chronic administration of PRV. All the results are summarized in Table 4.

Parameters and Unit	Control (Mean ± SEM)	PRV (Mean ± SEM)	% Change
Hemoglobin(g/dl)	12.060 ± 0.4770	11.300 ± .4159	Decrease 6.30
HCT(%)	42.980 ± 0.4994	38.940 ± 1.5500	Decrease 9.4
MCV(fl)	60.360 ± 0.3893	59.480 ± 0.4694	Decrease 1.46
MCH(pg)	17.520 ± 0.3261	17.280 ± 0.2267	Decrease 1.37
MCHC(%)	29.040 ± 0.6021	29.000 ± 0.1612	Decrease .14
RDW(%)	9.806 ± 0.0979	10.262 ± 0.1893	Increase 4.65
ESR(mm/h)	2.400 ± 0.2449	2.600 ± .2449	Increase 8.33
BT(sec)	48.000 ± 3.000	42.000 ± 3.000	Decrease 12.5
CT(sec)	219.000 ± 6.000	216.000 ± 3.6742	Decrease 1.37
MPV(fl)	3.816 ± 0.0645	3.868 ± 0.0656	Increase 1.36
PCT(%)	.1762 ± 0.00491	.1934 ± .03413	Increase 9.76
PDW(%)	14.420 ± 0.2395	14.380 ± 0.1624	Decrease 0.28

Table 4: Effect of punarnavasava (PRV) on hemoglobin, HCT, MCV, MCH, MCHC, and RDW in male rats.

Discussion

Hemotoxicological profile investigation is an essential part of determining the toxicity of any drug under experimentation on the constituents of the blood of an animal. The evaluation of risk is carried out by the analysis of hematological parameters to predict any abnormal toxicity sign in humans when tests involve rodents [28].In the current study, we have found noticeable changes in the hematologic parameters that include the possibility of the occurrence of leukemia.

White blood cells (WBC's) are responsible for the detection and destruction of pathogens that come into our body Blood leukocytes (WBC's) consist of a total of five cell lines that include neutrophils, monocytes, eosinophils, basophils, and lymphocytes [29,30].In the present investigation, we found a slight increase in the number of WBC. We have also noticed a high increase in the differential count of different WBCs is the sign of hypersensitivity, inflammation, and leukemia [31].Red Blood Cells (RBCs) help to produce, carry, and protect hemoglobin (Hb) for oxygen transport. Production of RBC's mainly happens extravascularly in the bone marrow parenchyma. Tissue hypoxia is the major stimulus for erythropoiesis and blood

hemoglobin concentration is the primary determinant of the degree of tissue oxygenation. Other factors affecting tissue oxygenation include cardiac output, pulmonary function, oxygen tension of inspired air, alterations in the oxyhemoglobin dissociation curve, and tissue blood distribution [32].In the current study, the count of RBC had slightly decreased which has less potential for the development of anemia. Platelets are the smallest formed element in the blood (~1-4/μm, ~3-15 fl) that derived from the myeloid stem cell. These are anucleate having a life span of about 4-10 days. In most species, counts range between 200,000-500,000/μL (rats, ~800,000/μL; mice>1,000,000/μL) [32,33]. The major function of platelets is to maintain hemostasis. They also play a role in coagulation (PF3, coagulation factors, clot retraction), as mediators of inflammation (chemotactic substances, vasoactive amines, and cationic proteins), and in phagocytosis of small particles and bacteria [32]. In our study, there is a slight increase of platelet count found which reflects the probability of producing myeloproliferative disorders, inflammation, infections, and cancers [34,35]. Hemoglobin (Hb) is the amount of oxygen-carrying protein contained within the RBCs [29]. And the concentration of Hb is reported as grams per deciliter of blood (g/dL). Since red cells are approximately 33% hemoglobin, the hemoglobin concentration of whole blood normally is about one-third of the HCT [36]. Packaging of Hb is beneficial due to several reasons because it helps to maintain the functional status, reduces turnover of Hb, and alleviates osmotic pressure. Besides oxygen transport, Hb also is the most important protein buffer in the blood (~6x the buffering capacity of plasma proteins) [32].Our study found a slight decrease in Hb concentration in RBC.

Neutrophils are another essential blood component that enters into the blood after production and maturation in the bone marrow. They have a circulating life of only 10-15 hours. They can easily transport into tissues of alveoli and lumen of the gut. Chemotactic factors produced at sites of inflammation direct migration of neutrophils from blood vessels into the tissue at those sites [37]. Corticosteroids tend to cause movement of neutrophils and release some cells from the marrow pool, thereby raising the neutrophil count in a blood sample which is called neutrophilia. Endotoxin tends to cause sequestration of neutrophils in the spleen, liver, and lung, thereby lowering the neutrophil count in a blood sample [38]. In our study, we have found an increased neutrophil differential count which indicated a possibility of internal inflammation due to chronic administration of PRV.

Eosinophils are produced in the marrow, circulate in the blood for a few hours, and migrate into tissues where they survive for several days. Increased production of eosinophils is mediated by factors produced by some activated T lymphocytes. Corticosteroids decrease blood eosinophil numbers but increase the marrow pool of eosinophils. Increased numbers of circulating eosinophils may be seen in hypersensitivity reactions, as with certain forms of parasitism and allergic conditions. We have found a very high increase in the absolute count of Eosinophils of the male rat. Basophils are normally of similar size to neutrophils or maybe slightly larger. An increased percentage was found in the current study. Lymphocytes can be the most numerous cell type in rodents. A statistically insignificant decrease in the absolute count of Lymphocytes of the male rat was obtained in this study. Lymphocytes, unlike the other leukocytes, are produced in lymphoid tissue rather than in marrow [39]. Changes in blood lymphocyte numbers usually reflect changes in distribution rather than changes in production or loss. A decrease in the absolute count of lymphocyte is termed as Lymphopenia. Lymphocytes can be transported to any kind of cell and can be easily attracted to damaged cells, pollens, or viruses [40]. In the current study, we found a slight decrease in the absolute count if Lymphocyte also a moderate reduction of whole WBC counts. Monocytes are produced in the bone marrow. They have some similarities with neutrophils such as production place (marrow), circulation into different blood cells or tissues when needed. Monocytes can further convert into macrophages. The corticosteroid effect differs among species. Factors produced at sites of inflammation can increase monocyte production. We have found an increased percentage of differential monocyte count which is termed as monocytosis that indicates the presence of stress or inflammation [39].

The HCT measures the ratio of blood volume occupied by RBCs and is reported as a percentage [32]. It can be calculated from MCV (mean cell volume) and RBC count. Therefore, any kinds of inaccuracies in the measurement of the MCV or RBC count, the HCT will reflect those inaccuracies [41]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are morphological measures and are useful in the classification of anemia. They can be calculated from the values of the HCT value, RBC count, and concentration of Hb. Therefore, the validity of the indices depends on the accuracy of the data required for calculation. MCV expresses the average RBC volume of a population of erythrocytes and is an indicator of "average" RBC size. MCV is increased (macrocytic cells) in anemias. Iron deficiency causes a decreased MCV MCH test helps diagnose the type of anemia. MCH expresses the weight of Hb in an average RBC in a population of cells. Iron deficiency causes decreased MCH due to decreased Hb production [32]. MCHC denotes the volume and character of the hemoglobin and is the most accurate of the red cell indices [42]. MCHC expresses the ratio of Hb weight to the volume of an average erythrocyte in a population of cells. MCHC is decreased in iron deficiency and reticulocytosis. The RDW is an index of the variation in cell volume within the red blood cell population. By measuring the RDW test, the sizes and shapes of the RBCs can be easily obtained. RDW is important because the more surface area the red cell has, the better it can hook onto and transport oxygen through the system [43]. The RDW may also be useful in monitoring the results of hematinic therapy for iron-deficiency or megaloblasticanemias [44]. Our study shows very slight changes in the MCV, MCH, MCHC, and RDW values which correlates the result of decreased red cell count and represents the presence of mild anemia in PRV administered male rat.

The ESR reflects the tendency of red blood cells to settle more rapidly in the face of some disease states or inflammation in the body. ESR can be affected by the alteration of size, shape, and count of RBC. Bleeding time (BT) test measures the time taken for blood vessel constriction and platelet plug formation to occur. As the BT test depends on the action of platelet and constriction of vessels, the formation of a clot is not allowed during this test. For clotting time test (CT), the enzyme thrombin must be generated from the plasma precursor prothrombin which then converts soluble fibrinogen into insoluble fibrin. However, thrombin production depends on the activation of several clotting factors and the presence of Ca^{++}. This process also requires different factors released by thrombocytes and other damaged tissues [45,46]. About 4-10 minutes is considered as the standard clotting time range. This study shows a slight increase in ESR value. Bleeding time and clotting time are decreased which correlates the slight reduction in platelet counts. In Mean Platelet Volume (MPV), individual size varies within a given sample. Large platelets have more clinical relevance in animals that are thrombocytopenic [34]. Plateletcrit (PCT) is the total packed volume of platelet in the

blood which includes the total number and size of the platelets. On the other hand, platelet distribution width (PDW) is defined as the variation in platelet size. PDW is a sign of active platelet release into blood and reflects the degree of anisocytosis. This study shows a very slight increase in the MPV and PCT value but a very negligible decrease in PDW.

Conclusion

In the present study, Punarnavasava (PRV) was found to be a mildly hemotoxic agent at a higher dose (40 ml/kg) after 45 days of chronic administration of the preparation. After the observations, there were statistically significant changes in Basophil and Lymphocyte count but showed negligible changes for other hematological parameters like RBC, Hemoglobin, Hematocrit, TC, DC, various erythrocytic parameters, platelet parameters, ESR and blood bleeding time and clotting times. Thus, PRV can be taken at a normal dose to treat ascites. We recommend future studies in a larger dose for determining chronic toxicity levels.

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