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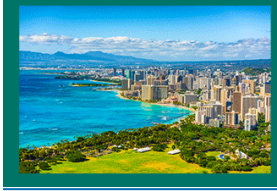

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The Benefits of Ethanolic Extract of *Plectranthus amboinicus* Lour Spreng on Rats Hematology Profile that Provided with Rhodamine-B

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Abstract. The objective of this study determines the effect of Plectranthus amboinicus L. Spreng ethanol extract on the hematological profile of rats exposed to Rhodamine-B. This study used 40 male rats that divided into eight groups consisted of five rats in each group. Group P0 was given 1% CMC. P1, P2, P3 groups were given 350, 700 and 1050 mg of ethanolic extract of *Plectranthus amboinicus* L. Spreng (EEP)/kg bw as preventive dose, and P4, P5, P6 groups were given 350, 700 and 1050 mg EEP/kg bw as a curative action, whereas Positive Control (PC) is given 980 mg of Rhodamine-B/kg bw. Preventive treatment was done by giving EEP from the 1st to 42nd day and Rhodamine-B was given from 21st to 42ndday. Whereas in curative treatment is done by inducting Rhodamine-B from the first to the 21st day and followed by EEP from the 22nd until the 42nd day. Parameters being observed were Erythrocyte, Total Leukocyte Count (TLC), Platelet (Plt), Hematocrit (Ht), Hemoglobin (Hb), MCH, MCHC and MCV.The results revealed that Rhodamine-B decreased the erythrocyte level significantly and there was no difference between the prevention and curative treatment. Platelets were decreased significantly in PC treatment but are similar to controls. The treatment in P4 and P5 showed significant increase in platelets level. The total leukocyte increased significantly in P6 treatment. The hematocrit (Ht) profile did not change in all treatments. The hemoglobin profile remains normal, however there was a significant decreased in PC and P6 treatment. MCV and MCH were increased significantly only in PC treatment. There was no difference in MCHC profile in all treatments of EEP and Rhodamine-B.

Keywords: Hematology profile, *Plectranthus amboinicus* Lour Spreng, Rhodamine-B

1. Introduction

In order to make food looks more attractive, the traders usually add some food colorings to their foods. Lately, due to the increasing price of food colorings and shorten expiry date, some traders replace them with synthetic dye known such as Rhodamine B (Rh-B). Rh-B is included to xanthene dyes which usually used for textile and papers industry as a dye for fabrics, cosmetics, oral cleaning products, and soap. Synthetic dyes are present in a very large number of products in daily use that are ingested (food, pharmaceuticals) or come into contact with our skin (cosmetics, textiles, leather, paper) and get into the body through the mouth, eyes, any cuts, or abrasions on the body. Rh-B contain Chlorine ,a halogen compound which is radical if it's found in the body because it can bind to proteins, lipids and DNA, potentially causes damage to the function of organs and blood in the body.

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Rh-B toxicity tests conducted on mice have proved the existence of a carcinogenic effect with the onset of local sarcoma in LD50 dose of 890 mg/kg [1]. Rh-B is a toxic substance if accumulated in the body and will cause various health problems. Therefore, the body requires daily food that can be used as an antioxidant.

Herbal medicines have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased. Researches have shown that oral ingestion of medicinal compounds or drugs can alter the normal range of hematological parameters. Antioxidants prevent or remove free radicals which can damage vital components of the cell. However, since reactive oxygen species have useful functions in cells such as redox signalling, the function of antioxidant system is not to remove oxidants entirely but instead to keep them at an optimum level. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidant compounds in food play a significant role as a health-protecting factor. Pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure [3]. With increased exposure to free radicals, the need for antioxidants in the human body becomes even more vital. Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk [4]. Many naturally occurring antioxidants-form plant sources-have been identified as free radical scavengers or active oxygen-scavengers [5, 6]. *Plectranthus amboinicus* (Lour.) Spreng is a tender fleshly perennial plant in the Lamiaceae family and native to southern and eastern Africa. The genus *Plectranthus* comprises of about 350 species distributed in Sub Saharan Africa, Australia, Asia, India, Madagascar and Pacific Islands [7]. Plectranthus amboinicus is used in the treatment of diseases such as digestive, genitourinary and respiratory disorders, skin infections and pain [8]. In India, it is used to treat malaria, cough, chronic asthma, bronchitis, colic, epilepsy and hiccups [9]. The pharmacological properties recorded include antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, fungi toxic, and anti-malarial activities [8].In Indonesia, especially in North Sumatra *Plectranthus amboinicus* leaves are used as vegetable, especially for mothers who just gave birth. In addition to increasing the breast milk production, the leaves also serve to heal wounds. Various vitamins are contained in the *Plectranthus amboinicus* like vitamin C, B12, vitamin E and beta carotene [10].The existence of various vitamins makes *Plectranthus amboinicus* as an antioxidant that can protect the body from various toxic substances disorders. In this research the toxic substance is Rh-B.

Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition [11]. The importance of hematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals [12].

The present study was aimed to know the effect of EEP on the hematology profile determined in albino rats that exposed to Rh-B. Hematological parameters determined includes the total number of erythrocytes and leukocytes (TLC), platelet (Plt), hemoglobin concentration (Hb), hematocrit (Ht), average cell volume (MCV), mean concentration hemoglobin (MCH), the average amount of hemoglobin in red blood cells (MCHC) in an automatic analyzer type BC Dray 2800 Vet Min.

2. Materials and Methods

2.1. Animals

Healthy adult albino male rats of 2 months in age and between 110 – 200 g in body weight were used in the study. The animals were maintained at room temperature on 12 h light – 12 h dark cycle. They were fed with PC 202 C pellet diet and tap water ad libitum. Rats were placed in plastic cages measuring 40 x 20 x 15 cm, at the top of the cages were equipped with wire cover. Each cage was

filled with chaff as the base and then one rat was placed per cage. Acclimatization was done for 7 days.

2.2. Ethanol Extract of Plectranthus amboinicus (EEP)

Ethanol extract from the plant leaves were processed using maceration method [13]. The extract was mixed with CMC 1% before given orally to the rats. Provision of EEP was carried out by maceration according to the method developed by [14] and [15]. In detail, the provision of EEP is described as follows. A total of 8 kg of fresh leaves was dried using oven with a temperature of 40oC to dry properly and brittle. Five hundred grams of dried leaves was smoothed with a blender to form powder. The leaf powder was placed in 2 containers with 250 g in each container, then 95% ethanol was added as much as 2000 ml/container. Leaf powder was macerated for 5 days and stirred once a day. The leaf extract was filtered using filter paper and ethanol was added to 3 litres, then allowed to stand for 2 days and re-filtered using Whatman filter paper. The extract obtained was then concentrated using a rotary evaporator.

2.3. Rhodamine-B (Rh-B)

Rhodamine-B (Sigma, Sigma Aldrich, Singapore) was dissolved with distilled water (30%) and administered orally using gavages. We chose an oral route caused by illegally food coloring use. The duration of administration of Rh-B in the treatment group according to the previous study which applied sub chronically toxicity tests of Rh-B for 20 days. The dose of Rh-B was 890 mg/kg bw [1].

2.4. Methods

This research consisted of preventive and curative treatment. Detailed design experiment can be seen in Table 1. Each was divided into five groups, they were control (P0) which CMC 1%was given, positive control (PC) where Rh-B 980 mg/kg bw was given, EEP with graded doses of 350 (P1), 700 (P2) and (P3) 1050 mg/kg bw. Preventive treatment was done by giving EEP from the 1st to 42ndday, and Rh-B was given from 21st to the 42nd day. The treatment for curative were 350 (P4), 700 (P5) and (P6) 1050 mg/kg bw. Curative treatment was done by induction with Rh-B from the first day to the 21st day, and then EEP was given from the 22nd until the 42nd day. On the 43rdday, there was blood taking through the decapitation for hematology analysis.

Table1. Design of Experiment

Group		Treatment
Control	P0	CMC 1% from the 1st to the 42nd day
Preventive	P1	On the 1st to 21nd day were given 350 mg EEP/kg bw On 22nd to 42ndday were given 980 mg Rh-B/kg bw
	P2	On the 1st to 21nd day were given 700 mg EEP/kg bw On 22nd to 42nd day were given 980 mg Rh-B/kg bw
	P3	On the 1st day to 21nd were given 1050 mg EEP/kg bw On days 22nd to 42nd day were given 980 mg Rh-B/kg bw
Curative	P4	On the 1st to the 21st day were given 980 mg Rh-B/kg bw On the 22nd to the 42nd day were given 350 mg EEP/kg bw
	P5	On the 1st to the 21st day were given 980 mg Rh-B/kg bw On the 22nd to the 42nd day were given 700 mg EEP/kg bw
	P6	On the 1st to the 21st day were given 980 mg Rh-B/kg bw On the 22nd to the 42nd day were given 1050 mg EEP/kg bw
Positive Control	PC	On the 1st through 21st day were given Rh-B 980 mg Rh-B/kg bw On the 22nd to the 42nd day were only given CMC 1%

2.5. Hematological Studies

Hematology parameters that included in this research were erythrocyte (RBC), Leukocyte (WBC), thrombocyte (PLT) count, Hemoglobin (Hb), MCV, MHC, and MHCH. On day 43rd the rats were decapitated and their blood were collected. Hematological data obtained using standard tools (ABX Micros 60).

2.6. Data analysis

Data are presented as mean ± SD and differences between groups were analyzed using one-way ANOVA. Post Hoc test was used if the ANOVA was significant. $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Erythrocytes

The hematopoietic system is one of the important indications to describe the physiological and pathological status of the body in humans and animals [16]. Assessment of red and white blood cell count, hematocrit and hemoglobin concentration is useful in determining the effect of some chemical substances on hematopoietic system. In Table 2, it can be seen that the erythrocyte profile of all treatments. Rh-B decreased the erythrocyte count significantly compared with EEP treatment and control. In both preventive and curative treatments the EEP maintains an erythrocyte count under normal circumstances. Erythropoiesis and hemoglobin synthesis require adequate supplies of vitamins B12, folic acid and mineral iron. Plectranthus amboinicus leaves contain many vitamins such as B12 as well as iron [10] that contribute to the maintenance of erythrocytes and hemoglobin, although was exposed to toxic Rh-B. The decrease in the amount of erythrocytes in PC treatment is due to Rh-B's own disturbance to possible erythropoiesis through interference with the kidneys.

Table 2. Profile of Erythrocyte, Platelet and Leukocyte

Treatment		Erythrocyte (10^{12}/L)	Platelets (PLT) (%)	Total Leukocyte Count (TLC) (10^9/L)
Control	P0	$7,56 \pm 0,62^b$	$604,00 \pm 66,41^a$	$10,45 \pm 1,05^a$
	P1	$7,51 \pm 0,93^b$	$630,50 \pm 50,88^b$	$11,19 \pm 2,14^a$
Preventive	P2	$7,17 \pm 0,87^b$	$660,00 \pm 30,10^b$	$11,99 \pm 1,04^a$
	P3	$7,75 \pm 0,27^b$	$585,00 \pm 54,64^a$	$11,16 \pm 2,94^a$
	P4	$7,27 \pm 0,26^b$	$641,00 \pm 60,02^b$	$12,59 \pm 3,63^b$
Curative	P5	$7,64 \pm 0,34^b$	$634,00 \pm 62,53^b$	$11,00 \pm 1,50^a$
	P6	$7,90 \pm 0,55^b$	$595,00 \pm 22,83^a$	$13,22 \pm 1,34^b$
Positive control	PC	$5,02 \pm 0,43^a$	$582,00 \pm 43,01^a$	$11,30 \pm 2,76^a$

Data are expressed as mean ± SD. Number of animals in each group is five.
Means which share the same superscript symbol(s) are not significantly different ($p< 0,05$)

3.*2. Platelets*

In this study, Rh-B decreased platelet significantly (Table 2). In both curative and preventive treatment, EEP 1050 mg/kg bw also decreased the platelet count significantly. However, in the treatment of 350 and 700 mg/kg bw both curative and preventive increase platelet count significantly. The number of platelets in this study is still lower than the results of [17] study on the administration of EEP 625 mg/kg bw giving platelet 688.20 ± 68.17 and 2500 mg/kg bw to 829.40 ± 16.44 (103 U/L). The increased platelets level in curative treatment in this study indicated that EEP was playing a

role in restoring the Rh-B induced disorders. Rh-B can increase blood coagulation; therefore platelets should be increased to anticipate thrombosis in blood vessels. In preventive treatment, EEP increased platelets for body defense. Platelets contribute to host defense as they recognize bacteria, recruit traditional immune cells to the site of infection and secrete bactericidal mediators. The body seeks to increase platelets for the purpose of body defense against toxic substances [18]. Increased platelets with EEP treatment in rats have been reported by [19].

3.3. Total Leukocyte Count (TLC)

The total leukocyte count can be seen in Table 2. The number of leukocytes increased significantly in the treatment of P6 and P4. In this study EEP with doses of 350 and 700 mg/kg bw worked in treating the toxic effects of Rh-B by increasing leukocytes. EEP, in this case, increased body resistance against toxic substances by increasing the number of leukocytes [17]. In the treatment of preventive leukocyte profiles tend to increase if compared to the controls treatment. In this case the EEP still increases the leukocytes after previously administered to prevent toxic effects of Rh-B. The ability of EEP as preventive and curative in this study is due to the presence of several components such as vitamin C, B12 and beta-carotene [10]

3.4. Hematocryte

The hematocrit (HCT) values together with erythrocytes and hemoglobin were parameters for determining the erythrocyte conditions. Increasing of HCT is one of indication for the occurrence of polycythemia, whereas decreasing of HCT is one indicator of anemia. In this study, the hematocrit value of P6 treatment decreased significantly if compared to all of the treatments (Fig 1).

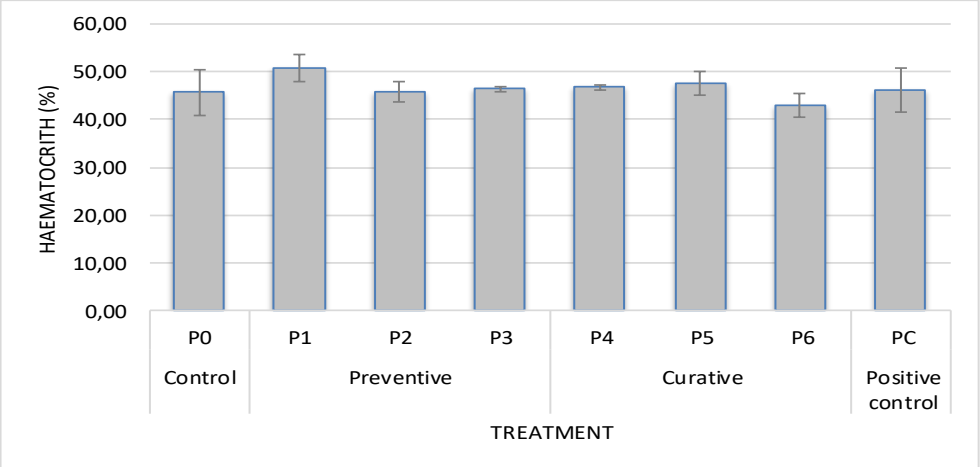

Figure 1. Hematocrit profile of rats treated with Rh-B in the preventive and curative treatment of EEP

The role of EEP as preventive and curative against the effects of Rh-B is to maintain hematocrit value. Although the curative treatment was lower in hematocrit but maintained within normal limits. Hematocrit levels are in line with hemoglobin (Fig1). The contribution of EEP to HCT enhancement is similar to EEP's contribution to erythrocytes and hemoglobin in the presence of some components found in EEP such as iron, vitamin B12 and some other nutrients [10]. The results of this study are in line with the [17] study and it is explained that the increase in hematocrit values due to EEP extract can stimulate the synthesis of erythrocytes.

3.5. Hemoglobin (Hb)

In this study, Hb increased significantly in treatment P1 (Fig. 2), whereas in PC treatment Hb decreased significantly as compared to the control and other preventive and curative treatment. The

increase of Hb in this study is in line with increasing hematocrit, MCV and MCH. The decreased of hemoglobin in PC treatment caused by Rh-B binding with Hb so that it decreased in circulation. This has been explained [20] that all four dyes studied form sufficiently strong interactions with hemoglobin to allow for the formation of potentially toxic interactions.

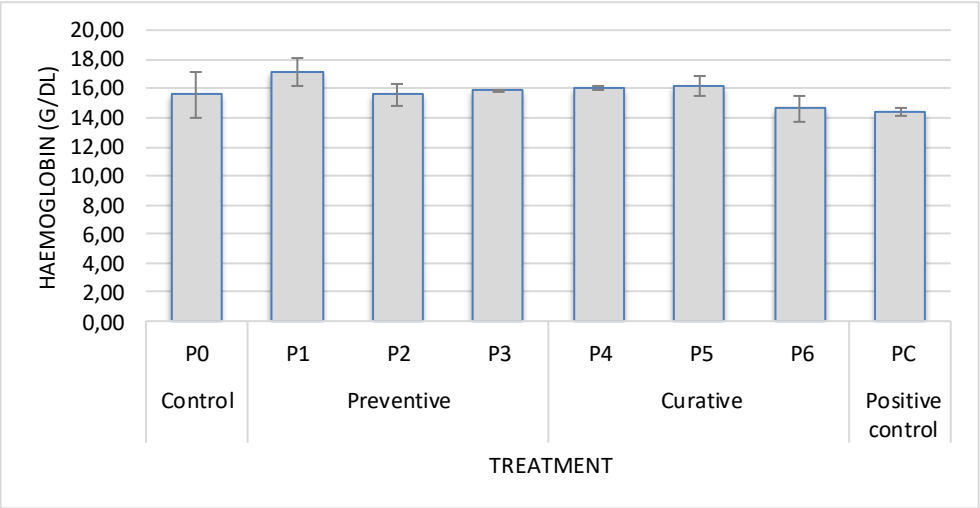

Figure 2. Hemoglobin profile of rats treated with Rh-B in the preventive and curative of EEP

Inside the body, dye molecules enter the blood stream, where they have been shown to have the ability to enter cells [21], including RBCs and interact with biomolecules present in the body, especially with proteins present in cells, tissue, and blood. In curative and preventative treatment Hb can be maintained in normal profile. This later fact explained that EEP plays a role in maintaining and restoring Hb levels. EEP also plays a stimulating role in erythrocytes, and with some EEP components such as Fe and beta-carotene [10] will be able to maintain Hb values in normal circumstances.

3.6. MCHC

Mean Corpuscular Hemoglobin (MCH) is one of the calculations that accompanied the characteristics of average size and content of erythrocytes. Normally, MCHC in male rats aged 17 months or more was 35.1 g / dl [22]. In this study, there was a significant decrease in MCHC in PC treatment as occurred in erythrocytes (Table 2) and Hemoglobin (Fig.2). Thus, the significant reduction in these parameters is an indication of severe anemia. In the values obtained in the hematological indices, slight fluctuations were recorded in the MCV and MCHC.

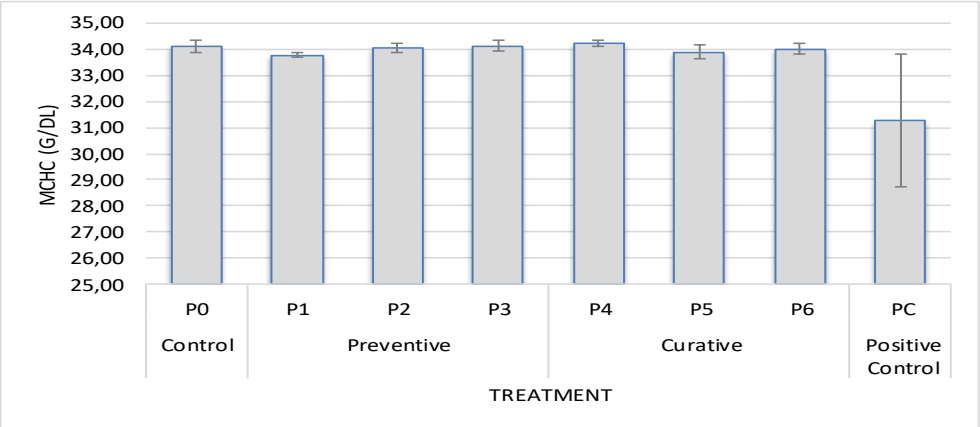

Figure 3. MCHC profile of rats treated with Rh-B in the preventive and curative of EEP treatment

MCH (mean corpuscular hemoglobin) is a type of examination to assess the average erythrocyte level. This examination is usually used as an indicator to see the level of human anemia. MCH is used to measure color index in erythrocytes in the blood. In this study, MCH increased significantly in PC treatment. EEP in curative and preventive treatment measures against the impact of Rh-B on MCH were keeping MCH levels normal. However, at treatment P6, MCH increased significantly. This is due to Rh-B binding to Hb [20]

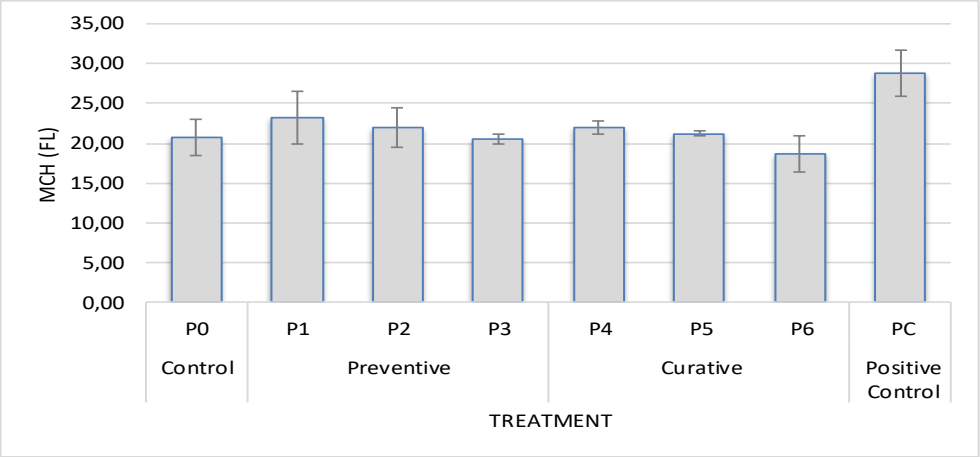

Figure 4. MCH profile of rats treated with Rh-B in the preventive and curative of EEP treatment

Means corpuscular volume (MCV) or mean erythrocyte volume is one of the parameters used to determine the type of anemia. MCV or mean corpuscular volume is used to measure the erythrocyte volume index in the blood. In this study, MCV increased significantly and was highest in PC treatment in line with MCH. The average volume of erythrocytes will increase if a substance binds to the erythrocytes. The dye that enters the blood will bind the hemoglobin [20].The curative and preventive action of the EEP can inhibit and release the bond thus the MCV becomes normal.

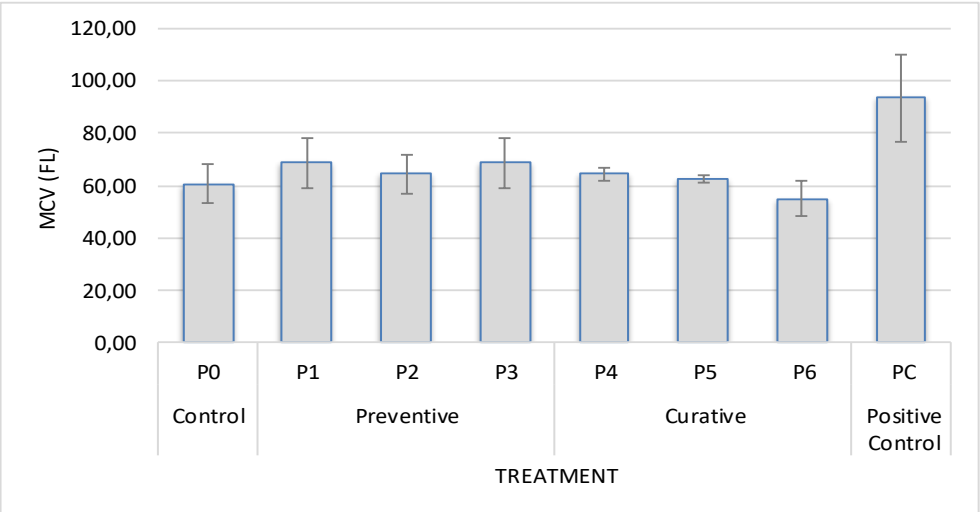

Figure 5. MCV profile of rats treated with Rh-B in the preventive and curative of EEP treatment

4. Conclusion

In both preventive and curative treatments the EEP maintains an erythrocyte count under normal circumstances. In both curative and preventive treatment, EEP 1050 mg/kg bb also decreased the platelet count significantly. However, in the treatment of 350 and 700 mg/kg bw both curative and preventive increased platelet count significantly. The number of leukocytes increased significantly in the treatment of P6 and P4. Hematocrit value of P6 treatment decreased significantly if compared to all of the treatments. Hb increased significantly in treatment P1, whereas in PC treatment Hb decreased significantly as compared to the control and other preventive and curative treatment. In this study, there was a significant decrease in MCHC in PC treatment. MCH increased significantly in PC treatment. As a preventive and curative EEP plays a role in maintaining MCH in normal profile despite exposure to Rh-B. MCV increased significantly in PC treatment as in MCH. The benefits of Ethanolic Extract *Plectranthus amboinicus* Lour Spreng on rats hematology profile that provided Rh-B was maintained normally and even increased or decreased according to the conditions to prevent or treat the effects of Rh-B.

5. Acknowledgement

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