Effect of *Diplaziumesculentum*(Retz.)Sw (Paco) extract against phenylhydrazine- induced anemia on Sprague Dawley rats

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Abstract: Anemia is considered to be the most common disorder of the blood. It is characterized by decreased number of red blood cells, deficient hemoglobin levels and decrease hematocrit value. Due to these hematologic alterations which had a great impact on health, many herbal medications are used in mitigating it. Studies show that leaf extract of *Diplaziumesculentum*(Retz.) Sw contains flavonoids and other polyphenols which may be used against many diseases. In this study, the effect of *D.esculentum* extract against phenylhydrazine-induced anemia on Sprague Dawley rats was determined. Hematologic parameters and morphological study of the blood cells were conducted to assess the activity of the extract against phenylhydrazine-induced oxidative stress.The lethal dose of the extract was found out to be greater than 225 mg/kg. Animal treated with only phenylhydrazine revealed a marked decline in red blood cell (RBC), hemoglobin concentration, and hematocrit level. On the other hand, the experimental group treated with the *D.esculentum* crude extract at a dose of 100mg/kg body weight and 200mg/kg body weight showed protection against the phenylhydrazine-induced oxidative stress. Morphological evaluations of the red blood cells of these animals through peripheral blood smear evaluation revealed recovery to hemolytic anemia by presence of normal shaped red blood cells after twelve day recovery period. In conclusion, the plant extract has the ability to protect the cells against the damaging effect brought about by phenylhydrazine administration.

Keywords: *Diplaziumesculentum*, anemia, phenylhydrazine, oxidative damage, red blood cell morphology

INTRODUCTION

The red blood cells or erythrocytes are the most common

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blood cells in the body. They are biconcave in shape and measuring about 6.0 to 8.0 microns in diameter. They play an important role in carrying oxygen in tissues and other organs in the body. Any unfavorable conditions like genetic disorders and parasitic infections may affect the morphology of the red blood cells causing decrease functionality and impose a certain hematologic disorder (Silva, Dao, Han, Lim, and Suresh, 2010).

Anemia is a blood disorder characterized by decreased number of circulating red blood cells and hemoglobin concentration in the body. As a result, the capability of the blood to carry oxygen throughout the body decreases. This hematologic abnormality is also associated with drug usage and toxicity, nutritional deficiencies and parasitic infections. Common indicator of anemia is decreased hemoglobin concentration which has values less than 13 g/dl in males and less than 12 g/dl in females. In tropical areas, anemia affects about 10 percent to 20 percent of the total population and this condition is commonly observed among geriatrics, pregnant women and infants. Anemia has also been reported to be associated with oxidative stress which an elevation of reactive oxygen species (ROS) causing damage to the red blood cells which result to hemolytic anemia. Oxidative stress is commonly associated with conditions like hereditary spherocytosis, thalassemia, sickle cell anemia, and G6PD (glucose-6-phosphate dehydrogenase) deficiency. It is also associated with some pathogenic diseases which can be life-threatening if left without medication (Koffour, Sam, Dadzeasah, Owiafe & Gyapong, 2012). According to de Benoist, McLean, Egli and Cogswell (2008), anemia affects about 24.8 percent of the world population. Pre-school aged children and pregnant women are the population groups which are greatly affected. In the Philippines, anemia affects about 19.5 percent of the total population which ranging from infants to elderly according to Arrolado and Osi (2010).

According to the study of Berger (2007), chemical exposure and drug usage are some factors that can induce anemia. Phenylhydrazine is a hydrazine derivative that can cause anemia through peroxidation of lipids in red blood cell membrane which results to its hemolysis. Moreover, the derivatives of phenylhydrazine are first used as anti-pyretic drugs but can cause adverse effects to the blood which made it toxic to the human body. These hematological toxicities involved destruction of red blood cells through oxidative stress and decrease levels of ATP (adenosine triphosphate) and glutathione (Roque, D'Anna, Gatti & Veuthey, 2008). It also causes formation of superoxide anion radicals and hydrogen peroxide that causes formation of Heinz bodies (Berger, 2007). These adverse effects often result to

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decrease hemoglobin level, circulating red blood cells, packed cell volume and impaired erythrocyte deformity (Shukla, Yadav, Singh, Bansode, and Singh, 2012). Other toxic effects of phenylhydrazine involved hypoxia, inflammation, alterations in the liver, kidney, central nervous system, autoimmune disturbances and cancer (Luangaram, Kukongviriyapan, Pakdeechote, KukongviriyapanandPannangpetch, 2006).

Because of the toxicities of phenylhydrazine, it became an interest in in-vitro studies for gathering information about hemolytic anemia. Many plant extracts were used as candidates in showing their effect in mitigating the said condition. In the study done by Akah, Okolo and Ezike (2009), the whole methanolic extract of *Brillantasianitens Lindau*was proven effective in reversing the effect of hemolytic anemia induced by phenylhydrazine. The authors also concluded that the vitamins, minerals and other phytochemicals present in the plant were the active components that mitigated the anemia. Similarly, Agbor, Oben, and Ngogang, (2005) had proven that the aqueous extract of *Hibiscus cannibinus* had hematinic property that provided treatment against hemolytic anemia. The study also presented that the aqueous extract of the plant had erythropoetic effect that causes rapid recovery of the experimental rats from the said condition. Likewise, a study conducted by Kouffor and his colleagues (2012) shows that the ethanolic root bark extract of *Carissa edulis* has also proven to provide treatment on the same condition. It shows that 300 and 1000 mg/kg of the plant extract were able to provide treatment to hemolytic anemia upon continued administration extract. The authors also claimed that the phytochemicals present in the plant had contributed in controlling the said condition.

In relation to the medicinal effect of various plant species stated above, *Diplaziumesculentum* (Paco) also had many benefits to human health. According to the Philippine Medicinal Plant (2014), *D. esculentum* is an edible fern that belongs to the family of Athyriaceae. It can grow an average height of 0.5 to 2.5 meters and usually thrive near banks of running streams (Philippine Medicinal Plant, 2014;Nair, Pradeesh, Nikhila, Sangeetha, Mini, andSwapna, 2013;Kaushik, Jijta, Kaushik, Zeray, Ambesajir,andBeyene, 2012). The plant is characterized by its black roots and leaves that are bipinnate or tripinnate. The leaves usually measures about 50 to 80 centimeters in length. Its pinnules are lance-shaped which measures about 5 centimeter in length and the sori are arranged in pairs in the side of the veins. The plant can be found widely in the Philippines, Polynesia and India and usually tribe on moist area like on the banks of running streams and river.Folkloric application of *D. esculentum* involved

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treatment against diarrhea, dysentery, stomach pain and can be used as insecticides. It was also believed that it can be used against fever, dermatitis, measles and given as tonic to women after childbirth (Shing, Wen, Wei, Chooi, Soo, andWeng, 2013; Amit and Singh, 2012).

Figure 1.*Diplaziumesculentum (*Paco) Plant

There were many studies and researches done on *D. esculentum* because of its beneficial effects to human. In the study conducted by Amit and Singh (2012), they showed that *D. esculentum* had anti-helminthic property in which the ethanolic extract of the plant causes paralysis and death of *Pheretimaposthuma*, an earthworm. In relation to this study, the said plant is also considered to be an effective antifungal agent. In vitro study shows that the methanolic extract obtained from the plant had inhibited the growth of certain fungi namely *Aspergillusniger, Rhizopusstolonifier*and *Candida albicans* (Zakaria, Sanduran, andSreenivasan, 2010). Similarly, an antibacterial study done by Shing et al. (2013) showed that the aqueous extract of *D. esculentum* had selective inhibitory activity against *Pseudomonas aeruginosa*. Moreover, the extract obtained from the plant had been proven to provide a lot of pharmacological benefits. In the study done by Kaushik et al.(2012), total anti-oxidant capability of the plant was measured using FRAP (ferric reducing ability of plasma). Aqueous extract of the plant was confirmed to have the greatest anti-oxidant property among other types of extract which in return proved its free radical scavenging power. The study also presented the ability of the plant as CNS (central nervous system) stimulant. Results showed that the aqueous extract of the plant increased the locomotor activity of experimental rats and its effect was compared to caffeine, a stimulant.

This study therefore aims to know the effect of *D. esculentum* extract in vivo on compensating the hemolytic anemia induced by phenylhyradrazine in rat models. This study determined the lethal dose of *D. esculentum* extracts being applied in rats. The assessment of the protective role of *D. esculentum* extract was accomplished by using

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hematological parameters such as red blood cell count, hemoglobin concentration and packed cell volume. Morphological assessment was completed using red blood cell indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular and hemoglobin concentration (MCHC) and by preparing peripheral blood smears. The mitigating effect of the extract based on the treatment done can be utilized to develop a low cost and readily available medication for those people affected with anemia.

MATERIALS AND METHODS

Plant

The leaves of *D. esculentum*were used in the study. The plant was collected from San Isidro, Lipa City. A sample of the plant specimen was submitted to the Department of Science and Technology Forest Products Research and Development Institute in the University of the Philippines-Los Baños, Laguna for authentication.

Drugs and Reagents

All drugs and reagents used were purchased at Bellman Laboratories in Quezon City.

Test Animals

A total of 42 Sprague Dawley rats of either sex weighing 150 - 200 grams and 30 albino mice were obtained from the Department of Pharmacology and Toxicology in the University of the Philippines College of Medicine, Ermita, Manila. The rats were housed in wire-mesh cages and were acclimatized for seven days. The rats were maintained at standard laboratory conditions (25±2°C and 30–60% relative humidity with a twelve hours light and dark cycle). They were allowed to have continuous access to food and water during the entire period of experimentation. All animal studies were carried out in accordance with the guidelines of the Philippine Association for Laboratory Animals Science (PALAS) and Bureau of Animal Industry (BAI) (Meena, Patidar & Singh, 2014; Arollado & Osi, 2010; Agbor, et al., 2005).

Preparation of the Crude Plant Extract

The powdered dried leaves were weighed (160 g) and soaked for 72 hours at room temperature in methanol with the ratio of 1:20 (w/v). The solutions were collected and filtered using cotton wool followed by Whatman No. 1 filter paper. This procedure was repeated three times. Then, the collected supernatant was pooled together and evaporated using a rotary vacuum evaporator at 65°C under reduced

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pressure. The evaporation of the methanolic extract of *D. esculentum*resulted a yield of 48.4 g (30.3%). The crude dried extract obtained was kept at 4°C prior to use (Kamisan, Yahya, Mamat, Kamarolzaman, Mohtarrudin, et al., 2014).

Phytochemical Study

Phytochemical screening of the crude extract was accomplished using the methods describe by Sumathy, Lachumy, Zakaria and Sasidharan (2011).

Test for Carbohydrate

Two millilitres of Molish's reagent and 2ml of concentrated sulphuric acid (H2SO4) was added to 2ml boiling methanolic extract. A reddish ring indicates the presence of carbohydrate.

Test for reducing sugar

Two milliliters of methanolic extract was added to boiling Fehling's solution for 5minutes. A brickred precipitate indicates the presence of reducing sugar.

Test for tannins

To 2ml of methanolic extract, 1ml of ferric chloride (FeCl3) was added and blue-black or greenish–black precipitate indicates presence of tannins.

Test for Saponins

One milliliter solution of the methanolicextract was diluted with distilled water to 20 ml and shaken in a test tube for 15 minutes. Development of stable foam suggests the presence of saponins.

Test for flavonoids

Magnesium ribbon and few drops of concentrated hydrochloric acid were added to 2ml of methanolic extract, pink or red color indicates the presence of flavonoids.

Test for alkaloids

Ten milliliters of ammoniacal chloroform solution was added to 2ml of methanolic extract. The extract was then treated with 10 drops of 10% sulphuric acid and tested with Meyer's reagent. Formation of white precipitate indicates the presence of alkaloids.

Test for phenols

To 2ml of methanol extract, 0.5ml of Folin-cicocalteau reagent

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and 2ml of 20% ofNa2CO3 was added and presence of bluish colour indicates the presence of phenols.

Test for anthraquinones

To 2ml of methanolic extract, 2ml of 10% NH4OH was added. A bright pink colour indicates the presence of anthraquinones.

Lethal Dose of Crude Extract (LD50)

The median lethal dose (LD50) of the crude extract was determined in mice intraperitoneally (*i.p.*). Briefly, mice of either sex were fasted overnight and the evaluation of the LD50 was carried out in two stages. In the first stage, three groups of three mice each were treated with the extract at doses of 10, 100 and 1000 mg/kg, *i.p.* in order to determine the range in which the LD50 falls. In the second stage, another four groups of three mice each were further treated with the extract at doses 140, 225, 370 and 600 mg/kg. Animals were observed for 24 hours after treatment for signs and symptoms of toxicity. The number of deaths in each group was recorded and the final LD 50 values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred) (Salawu, Chindo, Tijani, andAdzu, 2008).

Experimental Procedure

A total of 42 rats were used in the experiment. Rats were randomly divided into five groups with five animals each.

Group I: Saline Group (Normal Control)
GroupII:Phenylhydrazine(PHZ),10 mg/kg (Anemic Control)
Group III: Crude extract, 200 mg/kg
Group IV:Phenylhydrazine (PHZ)10 mg/kg and 100 mg/kg crudeextract
Group V:Phenylhydrazine (PHZ)10 mg/kg and 200 mg/kg crude extract

On the fourth day, blood samples were collected from the retro-orbital plexus vein of the rat's eyes in vials containing EDTA as the anticoagulant. These samples were evaluated for hematological parameters using a hematological cell counter. After four days, the administration of phenylhydrazine was discontinued in all the groups excluding group I and group II. The rest of the groups were treated with crude extract once a day, at the dose of 100 and 200 mg/kg body weight continuously up to next 12 days. The animals in group II served as the anemic control. Blood samples were collected on the sixteenth day and were evaluated for hematological parameters. On fourth and sixteenth

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days, slides of blood cells were prepared and stained for morphological analysis (Ranjana, Yogesh, Umesh, and Rajesh, 2012).

Hematological Parameters

Blood samples were collected (1 to 4 ml) through retro-orbital plexus vein in EDTA coated vials. The blood was collected after the induction of anemia with phenylhydrazine and after 12 days of treatment with the crude extract. Hematological parameters used in the study include red blood cell count(RBC), hematocrit (Hct), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using an automated hematology analyzer (Meenaet al., 2014; Agboret al., 2005).

Morphological Study of Blood Cells

The blood smear was prepared by placing a small drop of blood near the end of the slide and bringing the edge of another slide in contact with the drop and allowing the drop to bank evenly behind the spreader. The smear was fixed for at least 30 seconds in absolute methanol and the methanol was removed by tilting the slide. Staining solution (Wright stain) was applied and the slide was placed horizontally for 2 minutes. Aliquot of the buffer solution (Sorensen's buffer solution) was gently mixed without any of the stain running off the slide and without disturbing the surface of the blood film on the slide. Slide was left for three minutes and rinsed with the distilled water for 30 seconds. Slide was dried in a tilted position, covered with a glass cover slip and will be examined under light microscope (Ranjanaet al., 2012).

Statistical Analysis

Data obtained from animal experiments were expressed as mean ± S.E.M. (standard error mean). Statistical difference between the treated and the control group were evaluated by ANOVA, followed by the Dunnett's test to correlate the effect of the extract on haematological parameters and Independent Sample T-test to determine the effect of the extract between the fourth and sixteenth day. The p values <0.05 was considered statistically significant (Ranjana et al., 2012).

RESULTS AND DISCUSSION

I. Plant Extract

An oily, dark green extract was obtained from 3 kilos of dried *D. esculentum* leaves as seen on Figure 2.Total amount yield was 25 ml.

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Figure 2.*D. esculentum* extract

II. Phytochemical Analysis

The qualitative phytochemical analyses of the methanolic extract revealed positive results in the test for saponins, tannins and flavonoids. In the test for saponins, visible formation of foam was observed and lasted for about 10 minutes. This result agreed with the study ofGogoi and Zaman (2013), in which the leaves of *D. esculentum* containsaponins and other secondary plant metabolites like terpenoids and glycosides. The test for tannins also produced a positive result. A greenish-black precipitate was observed upon the addition of ferric chloride to the methanolic extract. The positive result obtained correlated with the study done by Asif andKhodadadi (2013). The study presented the phytochemical contents and medicinal uses of some tropical such as *D. esculentum*. Results obtained revealed the presence of tannins and other phytochemicals. The wound healing capabilities of the said plant metabolites were also proven.Flavonoids were also found to be present in D. esculentum extract producing a red color. Positive red color was produced upon the end of test as seen on Figure 3. Flavonoids are secondary plant metabolite that has many beneficial effects to the body. It had been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory,antitumor, and antioxidant activities. These health-promoting abilities of flavonoids made a decent interest in the field of medicine (Chae, Lee, and Park, 2013).

The said plant was negative for the qualitative test for carbohydrate, reducing sugar, phenols, alkaloids and anthraquinones. The results obtained were similar to that of Gogoi andZaman (2013), who revealed the presence ofterpenoids, glycosides, sterols, flavonoids, tannins and saponins.

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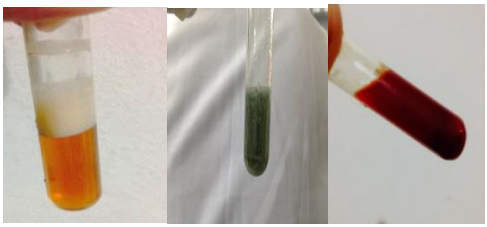

A. SaponinsB.TanninsC. Flavonoids

Figure 3. Phytochemical Results

III. Lethal Dose

Table 1 shows the lethal dose of the different of dosages of *D. esculentum*extract on albino mice. On the first stage, after the administration of the extract with doses of 10 mg/kg and 100 mg/kg it showed no toxic effects, while death occurred after administration of 1000mg/kg of the extract. This shows that within the range of 10 mg/kg to 100 mg/kg, the extract is safe to use.

Table 1
Lethal Dose of *D. esculentum* Extract on albino mice

	Dosages	Signs / Symptoms of Toxicity	No. of Deaths
First Stage	10 mg/kg	None seen	0
	100 mg/kg	None seen	0
	1000 mg/kg	Decreased respiratory function, skin discoloration	1
Second Stage	140 mg/kg	Decreased respiratory function	0
	225 mg/kg	Decreased respiratory function	2
	270 mg/kg	Decreased respiratory function	3
	600 mg/kg	Decreased respiratory function, skin discoloration	3

On the second stage, no death was observed after administration of the extract with a dose of 140 mg/kg. On the other hand, death occurred at the concentration of 225 mg/kg, 270 mg/kg and 600 mg/kg. The behavioral signs of toxicity exhibited by the mice that received 140 mg extract/kg and above were decreased respiratory rate, inactivity, increased abdominal contractions and change in skin coloration. However, the signs and symptoms observed were in contrast with the study of Devaki et al. (2012) in which increased locomotor

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activities was observed and no death occurred after administration of the extract. Additionally, intraperitoneal LD50 of the extract in mice was estimated to be 120 mg/kg. The results obtained implies that the concentration more than 225 mg/kg of extract have harmful effect if given higher than the suggested dosage obtained from the test.

IV. Hematological Examinations

Table 2 reveals the effect of crude extract of *D. esculentum* on hematologic parameters in phenylhydrazine-treated rats after four days treatment. As to red blood cell count, Group I obtained an initial red blood cell count of $6.41x10^6$uL which is higher than Group II with a red blood cell count of $3.65x10^6$uL. Group II demonstrated the lowest red blood cell count which indicates phenylhydrazine toxicity to the red blood cells. This result can be correlated to the study done by Berger (2007) in which exposure to certain chemicals such as phenylhydrazine can cause consequent destruction of red blood cells and cause secondary destruction of spleen and liver. Futhermore, Group III obtained an initial red blood cell count of $7.00x10^6$uL which is higher when compared to Group I. This result can be attributed to the bioactive compounds present in the plant. In correlation with the previous phytochemical analysis of Gogoi and Zaman (2013), it was found out that the plant contains flavonoids which have the ability to protect cells from oxidative damage. Moreover, Groups IV and V obtained initial red blood cell counts of $3.82x10^6$uL and $3.83x10^6$uL, respectively. The results obtained shows lower red blood cell count when compared to Group I. This is more likely due to the toxic effect of phenylhydrazine which still lingers to the test animals even when administered together with the crude extract after four days of treatment. Likewise, a study performed by Akah and colleagues (2009), in which primary administration of *Brillantasianitens*Lindau produced a partial recovery of animals from hemolytic damage produced by phenylhydrazine intoxication and it was also stated that it would take time to completely recover from the said condition.

As to hemoglobin, Group I obtained an initial hemoglobin level of 13.02g/dL which is higher compared to Group II with an initial hemoglobin level of 11.62g/dL. This means that phenylhydrazine has lowered the hemoglobin level of the test animals since a decrease in hemoglobin level below 12 g/dL signifies the presence of anemia (Akah et al., 2009). This is attributed to the ability of phenylhydrazine to denature hemoglobin and accumulation of free radicals causing formation of Heinz bodies (Berger, 2007). Futhermore, Group III produced an outstanding hemoglobin level of 14.08 g/dLwhichishigher compared to Group I.

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Table 2
Effect of Crude Extract of *Diplaziumesculentum*on Hematologic Parameters in Phenylhydrazine treated rats on 4th day

Groups	RBC	Hemoglobin	Hematocrit	MCV	MCH	MCHC
Group I (Normal)	6.41x10^6 uL	13.02g/dl	36.52%	57.06 fL	20.3 pg	355.6%
Group II (Anemic)	3.65 x10^6 uL	11.62g/dl	27.36%	76.34 fL	31.92 pg	435%
Group III (Crude extract)	7.00 x10^6 uL	14.08g/dl	40.44%	57.98 fL	20.1 pg	347.8%
Group IV (PHZ + 100 mg/kg Crude extract)	3.82 x10^6 uL	11.02g/dl	24.88%	65.58 fL	29.02 pg	443.6%
Group V (PHZ + 200 mg/kg Crude extract)	3.83 x10^6 uL	11.18g/dl	25.66%	67.94 fL	29.32 pg	433.8%

This implies that the crude extract has hematinic properties that increased the level of hemoglobin. In correlation, a study done by Koffour and colleagues (2012) suggested that *Carissa edulis* was able to reverse the effect of anemia. It was also stated in the study that the chemicals in plant such as polyphenols and terpenes were able to provide a protective effect to the red blood cells; thus, preventing the destruction of spectrin and the denaturation of hemoglobin. Moreover, Group IV and Group V obtained initial hemoglobin of 11.02 g/dL and 11.18 g/dL, respectively. This suggests that anemia still exist to the test animals since lower hemoglobin levels were obtained when compared to Group I. These results were further supported by the study done by Ranjana et al. (2012), which revealed that on the fourth day administration of *Tricosanthesdioca* crude extract, the animals were not able to fully recover from anemia since the effect of phenylhydrazine still lingers to the body system of the animals causing a delayed body homeostatic activity in reversing the said condition.

As to hematocrit, Group I obtained an initial hematocrit level of 36.52% which is higher compared to Group II with an initial hematocrit level of 27.36%. This specifies that phenylhydrazine has taken effect by lysing most of the red blood cells causing a decreased value in hematocrit level. In relation to this result, lowered values of hematocrit, red blood cell count and hemoglobin levels usually end to anemia according to Ashour (2014). In contrast, Group III got a hematocrit value of 40.44% which was higher than Group I. This only shows that thecrude extract of*D. esculentum* has beneficial effect to the

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blood system of the treated animals. In support, a study conducted by Gogoi and Zaman (2013) showed *D. esculentum* contains plant metabolites such as flavonoids which are known for its anti-oxidant properties. Moreover, decreased hematocrit values of 24.88% and 25.66%were further observed in Groups IV and V, respectively. This implies that the effect of phenylhydrazine intoxication still remains even when combined with the administration of the crude extract. In association, a study conducted by Sanni, Ibrahim, Esievo, and Sanni, (2005) stated that the extract of the *Khayasenegalensis* did not inhibit the anemia after simultaneous administration of phenylhydrazine for two days. It is also written in the study that the hematocrit value of the rats was restored after nine days of continuous treatment. This implies that the extract was able to protect the cells for further damage, but it would take time for the body to fully recover and reverse the effect of anemia.

Moreover, MCV, MCH and MCHC values were also observed in order to determine the presence of anemia after four days of initial treatment. From the results presented in Table 2, the mean cell volume of Group I obtained a value of 57.06 fL. This result is lower compared to Group II with a mean cell volume of 76.34%. This suggests the presence of anemia to the phenylhydrazine-treated rats since elevated level of MCV can be associated to the presence vitamin B12 deficiency and hemolytic anemia according to Ndem et al. (2013) and Kale and Aftab (2012). Futhermore, the test animals in Group III had an MCV result of 57.98 fL which was nearly the same with Group I. This can be attributed to the protective influence of the extract due to the presence of flavonoids since it did not cause a major increase in the MCV value of the experimental animals. This can be correlated done by Ranjana et al. (2012), in which the extract of *Trichosanthesdioica* provided a nearly similar MCV result to the normal control. On the other hand, Groups IV and V obtained initial mean cell volumes of 65.58 fL and 67.94 fL, respectively; which are still elevated when compared to Group I. This means that the effect of phenylhydrazine still remains. In addition, Group I exhibited initial MCH value of 20.3 pg which is lower compared to Group II with an initial MCH value of 31.92 pg.

In support, increased levels of MCH proved the presence of hemolytic anemia according to the study done by Ndem et al. (2013). Oppositely, Group III showed nearly same level of MCH with a value of 20.1 pg when compared to Group I. This only shows that *D. esculentum* extract has valuable protective influence to the blood and the body system which agreed to the study conducted by Gogoi and Zaman (2013). Lastly, Group I showed an initial MCHC value of 355.6% which was lower compared to Group II with an initial MCHC

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value of 435%. Furthermore, Groups IV and V produced MCHC valuesof 443.6 % and 433.8 % which are higher compared to Group I. In contrast, Group III provided a closer MCHC value of 347.8 % when compared to Group I. These results suggest that anemia still exist after four days of treatment and it would take time for the test animals to fully recover with the said condition which is parallel to the study done by Ranjana et al. (2012).

Futhermore, the effect of continued administration of *D.esculentum* extract up to 16th day in Sprague Dawley rats was summarized in Table 3. The red blood cell count was taken in consideration because it serves as a primary parameter in diagnosing different types of anemias. The result obtained from Group II showed lowered red blood cell count with the value of $4.23x10^6$ uL when compared to Group I. This indicates the ability of phenylhydrazine to cause anemia. The result obtained can be correlated to the study of Ndem, Otitoju, Akpanaiabiatu, Uboh, Uwah, and Edet (2013) in which phenylhydrazine causes denaturation of hemoglobin which results to decreased life span of red blood cells. It also causes accumulation of reactive oxygen species which often results to lysis of red blood cells.

Table 3
Recovery Period Observation of *D. esculentum* Crude Extract after Withdrawal of Phenylhydrazine from all groups (on 16th day)

Groups	RBC	Hemoglobin	Hematocrit	MCV	MCH	MCHC
Group I (Normal)	$5.74x10^6$uL	12.5g/dL	37.72%	66.26 fL	21.82 pg	332.0%
Group II (Anemic)	$4.23x10^6$uL***	11.84 g/dL	38.52%	91.28 fL****	27.96 pg****	307.0%**
Group III (Crude extract)	6.57 $x10^6$uL*	13.1 g/dL	38.84%	59.84 fL	20.06 pg	336.8%
Group IV (PHZ + 100 mg/kg Crude extract)	5.56 $x10^6$uL	13.9 g/dL**	41.1%	73.94 fL	24.96 pg*	338.2%
Group V (PHZ + 200 mg/kg Crude extract)	5.40 $x10^6$uL	13.36 g/dL	39.84%	74.04 fL	24.74 pg*	335.0%

*All values represent Mean ± SEM; (n=5), ns= not significant; ****=P<0.0001, ***=P<0.0005, **P<0.001, *P< 0.05 values are according to Oneway ANOVA followed by Dunnett test*

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On the other hand, an increased red blood cell count was obtained in Group III with the value of $6.57x10^6$ uL. The result obtained was even higher when compared to the control group. This indicates that*D.esculentum* extract had beneficial effect to the blood. In correlation a study conducted by Ndem et al. (2013) *Eremomastaxspeciosa* contains bioactive agents that may have brought about erythrocyte formation by stimulating the release of renal erythropoietin factor which stimulates the red bone marrow to produce more red blood cells. Moreover, results indicated that 100mg/kg crude extract increased the red blood cell count of Group IV from $3.82x10^6$ uL to $5.56x10^6$ uL after 12 more days of administration of extract. Group V also exhibited an elevation in red blood cell count from $3.83x10^6$ uL to $5.40x10^6$ uL. This implies that *D. esculentum* extract increases the red blood cell count of the rats after continued administration. This can be attributed to the presence of flavonoids and other bioactive agents present in the plant. In correlation, the study of Akah et al. (2009) revealed that *B. nitens* extract was able to elevate the red blood cell count of the test animals upon continued administration and was attributed to the presence of vitamins, minerals and other phytochemicals present in the plant.

Hemoglobin level was also checked, since low levels of hemoglobin in the blood can be associated with the presence of anemia. In Table 3, it was showed that Group II had nearly similar results from 11.64 g/dL after 4th day of treatment to 11.84 g/dLof hemoglobin on the 16^{th}day. Meanwhile, Group III showed a slight decrease on hemoglobin levels from 14.08 g/dL to 13.1 g/dL. As to Group IV and Group V, both showed an escalation of hemoglobin levels from 11.02 g/dLfrom the 4th day treatment to 13.9 g/dL and 11.18 g/dLto 13.36 g/dl, respectively. The results obtained from Table 3, in which hemoglobin levels of the animals treated with the 100 mg/kg and 200 mg/kg of the extract were higher when compared to the control group. This indicates that treatment of *D. esculentum* crude extract can counter the adverse effect of phenylhydrazine in hemoglobin concentration. In correlation to the results presented, the study done by Agbor et al. (2005) stated that two weeks administration of *Hibiscus cannabinus* extract caused an increased in hemoglobin concentration even higher in the control group. The said extract also increases the other hematologicalparameters. The study also presented that *H.cannabinus* extract had hematinic property that mitigate the effect of phenylhydrazine toxicity which caused the speedy recovery of animals from anemia.

Hematocrit is always part of a complete blood count. It measures the percentage of volume of whole blood that made up the

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red blood cells. Low hematocrit values can also be associated with the presence of anemia or destruction of red blood cells. To correlate, increased values were observed in Groups I and II from 36.52% to 37.72% and 27.36% to 38.52%, respectively after continued treatment up to 16thday. Further, the values for Groups IV and V all augmented from 24.88% to 41.1% and 25.66% to 39.84%, respectively. Protective influence of the crude extract was again apparent based from the results. In support, the study conducted by Akah and colleagues (2009) presented that *Brillantasianitens* extract caused an escalation of hematocrit values and other blood indices of phenylhydrazine-treated animals. The studyalso presented that biochemical agents present in the plant causes increased production of red blood cells; thus, recovery of rats from hemolytic anemia.

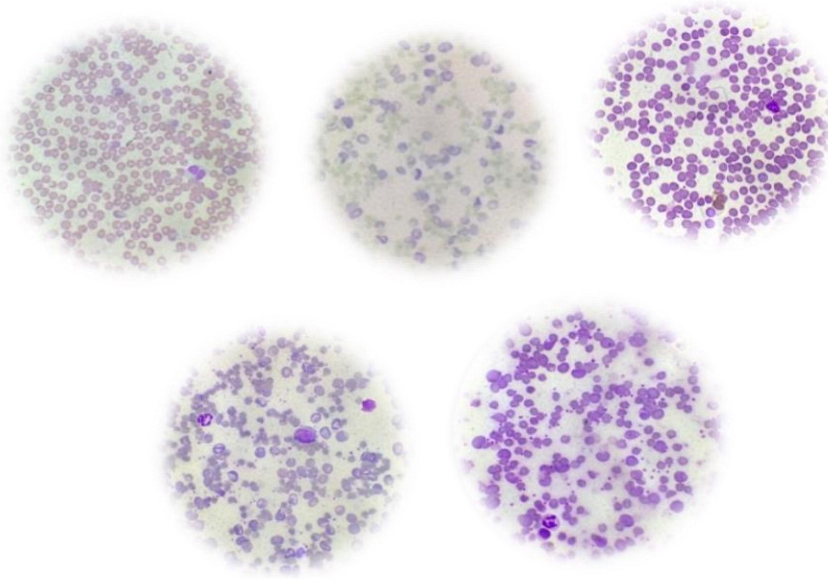

Figure 4. Microscopic Examination of red blood cell of Sprague Dawley rats

The treatment given to Group V showed lower RBC count, but an elevated level of hemoglobin and hematocrit when compared to Group I. This can be attributed to the increased dosage of the extract which causes mild alterations in hematological parameters of the experimental animals. As support, a related study done by Devaki, Beulah, Akila, andGopalakrishnan (2012) presented that higher dosage

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of *Passifloraedulis* extract causes slight decreased values of blood parameters of rats. However, results obtained from Group V for the said blood parameters were increased when compared to the anemic control.

Meanwhile, the peripheral blood smear evaluation and MCV, MCH and MCHC values were observed to determine the presence of anemia after 16 days recovery period. In Table 3, the mean cell volume was increased in Group II with the value of 91.28 fL when compared to Group I. This indicates the presence of anemia since increased MCV is associated with megaloblasticanemias and chronic hemolytic anemias according to the study of Ndem et al. (2013). In addition, the groups treated with *D. esculentum* extract showed decreased MCV values when compared to Group II as shown in Figure 4.This proves that the bioactive agents present in *D. esculentum*extract were able to improve the condition of the test animals from anemia. Furthermore, Group IIalso exhibited an increase MCH values when compared to Group I. An increased level of MCH is an indicative of intravascular hemolysis according to Ndem et al. (2013). On the other hand, Group IV and Group V showed lower levels of MCH with a value of 24.96 pg and 24.74 pg when compared to Group II, although higher when compared to Group I. This shows that the extract had ameliorating effect which reduced the intravascular hemolysis caused by phenylhydrazine which agreed to the study of Ndem et al. (2013). Lastly, the MCHC level of Group II was lower when compared to Group I. In addition, animals administered with varying dose of the same extract exhibit increased MCHC levels. This further suggests the hematoprotective activity of the extract which is in accordance to the study done by Ndem et al.(2013).

Moreover, Table 4 shows the multiple comparison of Groups I to V on red blood cell count. Red blood cell count comparison of Group I to Group II produced a p-value of 0.000 which is highly significant since it is less than 0.05 level of significance. On the other hand, Groups III, IV and V when compared to Group I produced p-values of 0.065, 0.971 and 0.753, respectively which are greater than 0.05 level of signifance which means that there is no significant difference in the values. Red blood cell count comparison of Group II to Group III produced a p-value of 0.000 which is highly significant since it is less than 0.05 level of significance. The same is for Group IV and Group V when compared to Group II produced p-values of 0.001 and 0.005, respectively which are less than 0.05 level of significance. This signifies that phenylhydrazine has truly able to significantly lower the red blood cell count of the test animals. Moreover, Group III when compared Group IV produced p-value of 0.068 which is greater than 0.05 level of significance which means there is no significant difference

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with the value. Group V when compared to Group III obtained a p-value of 0.005 which is less than 0.05 level of significance which means significant difference exists with the values. Furthermore, Group IV when compared to Group V produced a p-value of 0.976 which is not significant since it is greater than 0.05 level of significance.

Table 4
Multiple Comparison of Groups I to V on RBC count

Control	Groups	p-values	Interpretation
Group I Vs	Group II (Anemic)	0.000	Highly significant
	Group III (Crude)	0.065	Not significant
	Group IV(PHZ+100 mg/kg crude extract)	0.971	Not Significant
	Group V(PHZ+200 mg/kg crude extract)	0.753	Not Significant
Group II Vs	Group I (Normal)	0.000	Highly significant
	Group III (Crude)	0.000	Highly significant
	Group IV (PHZ+100 mg/kg crude extract)	0.001	Significant
	Group V (PHZ+200 mg/kg crude extract)	0.005	Significant
Group III Vs	Group I (Normal)	0.065	Not significant
	Group II (Crude)	0.000	Highly significant
	Group IV (PHZ+100 mg/kg crude extract)	0.068	Not Significant
	Group V(PHZ+200 mg/kg crude extract	0.005	Significant
Group IV Vs	Group I (Normal)	0.971	Not Significant
	Group II (Anemic)	0.001	Significant
	Group III (Crude)	0.068	Not Significant
	Group V (PHZ+200 Mg/kg crude extract)	0.976	Not significant
Group V Vs	Group I (Normal)	0.753	Not significant
	Group II (Anemic)	0.005	Significant
	Group III (Crude)	0.005	Significant
	Group IV (PHZ+100 mg/kg crude extract)	0.976	Not significant

Legend: Significant at p-value < 0.05

There is no significant difference obtained when Group IV and Group V was compared to Group I; thus, it indicates the same result

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statistically. This implies that *D. esculentum* extract had provided protective influence to the red blood cells against phenylhydrazine toxicity after 16 days of treatment which may be attributed to the phytochemicals present in the plant. Likewise in the study done by Gogoi in Zaman in 2013 showedthat*D. esculentum*extract contains flavonoids that protect cells from oxidative damage.

Table 5 shows the multiple comparison of Groups I to V on hemoglobin. Statistical data shows that Group I compared to Group II produced a p-value of 0.002 which is significant since it is less than 0.05 level of significance. Groups III, IV and V when compared to Group I produced p-values of 0.581, 0.268 and 0.245, respectively which are greater than 0.05 level of significance which indicate no significant difference with the values.

Table 5
Multiple Comparison of Groups I to V on Hemoglobin

Control	Groups	p-values	Interpretation
Group I Vs	Group II (Anemic)	0.002	Significant
	Group III (Crude)	0.581	Not significant
	Group IV(PHZ+100 mg/kg crude extract)	0.268	Not Significant
	Group V(PHZ+200 mg/kg crude extract)	0.245	Not significant
Group II Vs	Group I (Normal)	0.002	Significant
	Group III (Crude)	0.038	Significant
	Group IV (PHZ+100 mg/kg crude extract)	0.000	Highly significant
	Group V (PHZ+200 mg/kg crude extract)	0.009	Significant
Group III Vs	Group I (Normal)	0.581	Not significant
	Group II (Crude)	0.038	Significant
	Group IV (PHZ+100 mg/kg crude extract)	0.309	Not significant
	Group V(PHZ+200 mg/kg crude extract)	0.966	Not significant
Group IV Vs	Group I (Normal)	0.268	Not Significant
	Group II (Anemic)	0.000	Highly significant
	Group III (Crude)	0.309	Not significant
	Group V (PHZ+200 Mg/kg crude extract)	0.671	Not significant
Group V Vs	Group I (Normal)	0.245	Not significant
	Group II (Anemic)	0.009	Significant
	Group III (Crude)	0.966	Not significant
	Group IV (PHZ+100 mg/kg crude extract)	0.671	Not Significant

Legend: Significant at p-value < 0.05

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Meanwhile, the comparison of Group II to Group III, IV and V produced p-values of 0.38, 0.000 and 0.009, respectively which are less than 0.05 level of significance which means that there is significant difference in the values. This indicates that phenylhydrazine has significant lowering effect on the level of hemoglobin of the test animals. On the other hand, Group III compared to Groups IV and Group V produced p-values of 0.309 and 0.966, respectively which indicate no significant difference since it is greater than 0.05 level of significance. Moreover, Group IV when compared to Group V produce a p-value of 0.671 which is greater than 0.05 level of significance which indicate no significant difference with the values. The results obtained states that there is no significant difference in Groups III, IV and V when compared to Group I. This implies that the extract has able to increase the hemoglobin levels of the test animals after 16 days of treatment against phenylhydrazine induced anemia. It may due to the bioactive compounds and nutrients present in the plant. These results can be correlated to the study performed by Kaushik et al. (2012) in which *D. esculentum* plant has good anti-oxidant properties and a good source of iron necessary for increasing hemoglobin levels.

Table 6
Multiple Comparison of Groups I to V on Hematocrit

Control	Groups	p-values	Interpretation
Group I vs	Group II (Anemic)	0.048	Significant
	Group III (Crude)	0.928	Not significant
	Group IV(PHZ+100 mg/kg crude extract)	0.152	Not significant
	Group V (PHZ+200 mg/kg crude extract)	0.566	Not significant
Group II vs	Group I (Normal)	0.048	Significant
	Group III (Crude)	0.009	Significant
	Group IV (PHZ+100 mg/kg crude extract)	0.038	Significant
	Group V (PHZ+200 mg/kg crude extract)	0.027	Significant
Group III vs	Group I (Normal)	0.928	Not significant
	Group II (Crude)	0.009	Significant
	Group IV (PHZ+100 mg/kg crude extract)	0.506	Not significant
	Group V(PHZ+200 mg/kg crude extract)	0.951	Not significant
Group IV vs	Group I (Normal)	0.152	Not significant
	Group II (Anemic)	0.038	Significant
	Group III (Crude)	0.506	Not significant
	Group V(PHZ+200 mg/kg crude extract)	0.894	Not significant
Group V vs	Group I (Normal)	0.566	Not significant
	Group II (Anemic)	0.027	Significant
	Group III (Crude)	0.951	Not significant
	Group IV (PHZ+100 mg/kg crude extract)	0.894	Not significant

Legend: Significant at p-value < 0.05

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Table 6 shows the multiple comparison of of Groups I to V on hematocrit. Statistical data shows that Group I compared to Group II produced a p-value of 0.048 which is significant since it is less than 0.05 level of significance. Groups III, IV and V when compared to Group I produced p-values of 0.928, 0.152 and 0.566, respectively which are greater than 0.05 level of significance which indicate no significant difference with the values.

Meanwhile, the comparison of Group II to Groups III, IV and V produced p-values of 0.009, 0.038 and 0.027, respectively which are less than 0.05 level of significance which means that there is significant difference in the values. This indicates that phenylhydrazine has significant lowering effect on the hematocrit levels of the test animals. On the other hand, Group III compared to Groups IV and Group V produced p-values of 0.506 and 0.951, respectively which indicate no significant difference since it is greater than 0.05 level of significance. Moreover, Group IV when compared to Group V produce a p-value of 0.894 which is greater than 0.05 level of significance which indicate no significant difference with the values. The results obtained revealed that there is no significant difference in Groups III, IV and V when compared to Group I. This implies that the extract has able to increase the hematocrit levels of the test animals after 16 days of treatment which is a good indicator of recovery from hemolytic anemia. This may be due to certain phytochemicals such as flavonoids which are good anti-oxidants. Likewise, a study done by Gogoi and Zaman (2013) showed that *D. esculentum* plant contains good amount of the said phytochemical.

Table 7 shows the multiple comparison of of Groups I to V on red cell indices. As to MCV, statistical data show that Group I compared to Group II produced a p-value of 0.000 which is less than 0.05 level of significance which means there is significant difference in the values. Group III, IV and V when compared to Group I produced p-values of 0.423, 0.256 and 0.246, respectively which is greater than 0.05 level significance which means there is no significant difference in the values. On the other hand, Group II when compared to Groups III, IV and V showed significant difference due to p-values of 0.000, 0.001 and 0.001, respectively which is less than 0.05 level of significance.This indicates that phenylhydrazine has significantly increased the MCV values of the test animals.Moreover, Group III when compared to Group IV and Group V provided p-values of 0.368 and 0.387, respectively showed no significance since the p-value is greater than 0.05 level of significance. Group IV when compared to Group V also shows no significance due to the p-value of 1.000 which is greaterthan 0.05levelof significance. This implies that the extract has

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able to provide ameliorating effect which reduced hemolysis brought by phenylhydrazine toxicity. This is may be due to the bioactive agents and nutrients present in the plant. Likewise, to the study performed by Kale and Aftab (2012) showed that *D. esculentum* plant has components that can reverse oxidative stress brought by certain chemicals.

Table 7
Multiple Comparison of Groups I to V on Red Cell Indices

Control	Groups	MCV p-values	MCH p-values	CHC p- values
Group I Vs	Group II (Anemic)	0.000*	0.000*	0.012*
	Group III (Crude)	0.423	0.359	0.953
	Group IV (PHZ+100 mg/kg crude extract)	0.256	0.263	0.890
	Group V (PHZ+200 mg/kg crude extract)	0.246	0.268	0.992
Group II Vs	Group I (Normal)	0.000*	0.000*	0.012*
	Group III (Crude)	0.000*	0.000*	0.002*
	Group IV (PHZ+100 mg/kg crude extract)	0.001*	0.032*	0.002*
	Group V (PHZ+200 mg/kg crude extract)	0.001*	0.019*	0.004*
Group III Vs	Group I (Normal)	0.423	0.359	0.953
	Group II (Crude)	0.000*	0.000*	0.002*
	Group IV (PHZ+100 mg/kg crude extract)	0.368	0.260	1.000
	Group V (PHZ+200 mg/kg crude extract)	0.387	0.271	0.999
Group IV Vs	Group I (Normal)	0.256	0.263	0.890
	Group II (Anemic)	0.001*	0.032*	0.002*
	Group III (Crude)	0.368	0.260	1.000
	Group V(PHZ+200 mg/kg crude extract)	1.000	0.999	0.989
Group V Vs	Group I (Normal)	0.246	0.268	0.992
	Group II (Anemic)	0.001*	0.019*	0.004*
	Group III (Crude)	0.387	0.271	0.999
	Group IV (PHZ+100 mg/kg crude extract)	1.000	0.999	0.989

*Legend: *Significant at p-value < 0.05*

As to MCH, statistical data show that Group I compared to Group II produced a p-value of 0.000 which is less than 0.05 level of significance which means there is significant difference in the values. Group III, IV and V when compared to Group I produced p-values of 0.359, 0.263 and 0.268, respectively which is greater than 0.05 level significance which means there is no significant difference in the values. On the other hand, Group II when compared to Groups III, IV and V showed significant difference due to p-values of 0.000, 0.032 and 0.019, respectively which is less than 0.05 level of significance. Moreover, Group III when compared to Group IV and Group V provided p-values of 0.260 and 0.271, respectively showed no

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significance since the p-value is greater than 0.05 level of significance. Group IV when compared to Group V also shows no significance due to the p-value of 0.999 which is greater than 0.05 level of significance.

As to MCHC, statistical data show that Group I compared to Group II produced a p-value of 0.012 which is less than 0.05 level of significance which means there is significant difference in the values. Group III, IV and V when compared to Group I produced p-values of 0.953, 0.890 and 0.992, respectively which is greater than 0.05 level significance which means there is no significant difference in the values. On the other hand, Group II when compared to Groups III, IV and V showed significant difference due to p-values of 0.002, 0.002 and 0.004, respectively which is less than 0.05 level of significance. Moreover, Group III when compared to Group IV and Group V provided p-values of 1.000 and 0.999, respectively showed no significance since the p-value is greater than 0.05 level of significance. Group IV when compared to Group V also shows no significance due to the p-value of 0.989 which is greater than 0.05 level of significance. The results obtained states that there is no significant difference in Groups III, IV and V when compared to Group I. This implies that the extract was able to improve the condition of test animals after 16 days of treatment against phenylhydrazine induced anemia since non-significance of the result upon comparison with Group I signify the effect of the extract is the same. This is may be due to the bioactive compounds and nutrients present in the plant. Similarly, insignificant changes in MCHC together with MCH values, signify the absence of anemia which is stated in the study done by Oyedeji and Bolarinwa (2013).

Table 8
Comparison on the Effect of *D. esculentum* Crude Extract on Hematological Parameters

Hematological Parameter	p-value	Interpretation
RBC Count	.000	Significant
Hemoglobin	.001	Significant
Hematocrit	.182	Not significant
MCH	.000	Significant
MCV	.000	Significant
MCHC	.001	Significant

.*Significant at p-value <0.05

Table 8 reveals the statistical analysis of the hematological parameters when treated according to group. Statistical evaluation showed that the red blood cell count (RBC), hemoglobin, MCH, MCV, MCHC values were significant with p-values of .000, .001, .000, .000,

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.001, respectively. This means that there is significant difference observed across each group since the p-values are less than 0.05 level of significance. The significant increase in red blood cell count, hemoglobin, MCH, MCV, MCHC proves protective influence on *D. esculentum* crude extract against phenylhydrazine–induced anemia. In correlation with the study accomplished by Ndem et al. (2013), such result can be hypothesized to be attributed to the presence of phytochemicals in *D. esculentum* extract such as flavonoids which have favorable biological effect, that is, its anti-oxidant activity, thereby, reversing the oxidative damage brought about by the formation of reactive oxygen species and free radicals that damage blood cells associated with phenylhydrazine intoxication (Chae, Lee, and Park (2013); Cushnie and Lamb (2005). However, the hematocrit level obtained a p-value of .182 which is greater than 0.05 level of significance, indicating that it does not show significant difference and signifies that the effect is the same.

Table 9

Comparison on the Effect of *D. esculentum* Crude Extract in 4th and 16th day

Hematological Parameters	p-value	Interpretation
RBC count	0.123	Not Significant
Hemoglobin	0.044	Significant
Hematocrit	0.000	Highly Significant
MCV	0.017	Significant
MCH	0.076	Not Significant
MCHC	0.000	Highly Significant

Significant at p-value <0.05

Table 9 reveals the comparison of the effect of *D. esculentum* extract on the 4th and16th day of treatment. It was observed that there were significant differences that exist on the test conducted for the 4th day and 16th day. This was seen on hemoglobin (p-value <0.05), hematocrit (p-value <0.05), MCV (p-value < 0.05) and MCHC (p-value < 0.05). This indicates that the animals were able to recover in hemolytic anemia upon continued administration of *D. esculentum* extract. These results can be correlated to the study done by Ranjana et al. (2012), in which upon continued administration of *Tricosanthesdioca* extract was able to improve the conditions of the albino rats from the same blood disorder. However, insignificant result was obtained upon the comparison of red blood cell count and MCH. These imply that the effect of the extract were the same throughout the experiment in these blood indices. In correlation to the study done by Oyedeji andBolarinwa (2013), insignificant red blood cell results

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indicate that there is no destruction of red blood cells which may signify the recovery of animals from anemia. Furthermore, insignificant changes in MCH signify the absence of macrocytic anemia, since increased values indicate the presence of this type of anemia.

CONCLUSION

The above mentioned results show that the administration of*D. esculentum* extract prior to phenylhydrazine intoxication can increase the red blood cell count, hemoglobin and hematocrit values of the experimental rats. The lowest concentration of the extract at 100 mg was able to provide a protective influence against phenylhydrazine toxicity in the blood; thus, mitigating its effect which was supported by the evaluation of morphological appearance of cells done after 16 days of treatment. The crude extract was also able to preserve the normal morphology of red blood cells. Hence, *D.esculentum* may be used as a potential treatment for phenylhydrazine-induced anemia.

RECOMMENDATION

Advanced pharmacological evaluation by using extracts from other parts of *D. esculentum* may be used to further explore its benefits and resistance against the toxicity on the different blood parameters brought by phenyl hydrazine. Additional evaluation of the substances present in the plant must be given focus in order to know the spectrum of its therapeutic effect. Isolation of the active phytochemical components that is responsible for its protective effect would also be helpful in the evaluation of its therapeutic benefits.

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