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Screening of water distilled *Rosa damascena* Mill. flowers as hematopoietic agent in an animal model

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Abstract: Pancytopenia is classified as low blood cell count. Low levels of hemoglobin, red blood cells, white blood cells and platelets are indicative of pancytopenic state. This pancytopenic state can be treatment (drug) or disease induced. Conventional approaches available to treat pancytopenia are usually associated with many undesirable adverse effects, are costly and parenterally administered. Interest in natural products has significantly increased due to their ability to stimulate cellular components of immune system. This study is designed to investigate the hematopoietic i.e. erythropoeitic, leucopoietic and thrombopoeitic potential of water distilled flowers of *Rosa damascena* Mill.

Keywords: Hematopoietic, hemoglobin, red blood cells, white blood cells, platelets.

INTRODUCTION

Natural products have been widely used globally either to improve health or to treat unhealthy conditions. These natural products are still serving as a major source for discovery of novel drugs and compounds. Numerous conventional medicines have been derived from natural sources such as paclitaxel (an anti-neoplastic agent) from yew tree *taxus*, metformin (drug used in the treatment and management of type II diabetes) from *Galega officinalis*, artemisinin (a standard anti-malarial drug) from *Artemisia vulgaris*, Galantamine (an anti-dementia drug) from *Galanthus nivalis* etc. (Zahn *et al*., 2019). Natural products and their synthetic derivatives have been successfully used in clinical practices for the treatment and management of both acute and chronic illnesses. Still a massive research is going on to explore new therapeutic agents from these natural medicinal plants (Khan & Ahmad, 2019).

Rosa damascena Mill. is commonly known as Gul-e-Surkh, Gul-e-Muhammadi, Otto rose, Damascus rose, Ward-e-Ahmar and Gulab in native regions. It belongs to the family recognized as "King of Flowers" i.e. Rosaceae. It is widely cultivated in different regions of the world including Middle East, Europe, China, North America and India (Ali *et al*., 2016). Flower of *Rosa damascena* Mill. has been extensively used in food, perfume and medicine industries. Traditionally it is considered as an important medicinal plant due to its beneficial effects found in various diseases such as cardio-vascular diseases, gastrointestinal disorders, inflammatory processes, wound healing, skin diseases, mental disorders, pregnancy related issues and menstrual irregularities (Davoodi *et al*., 2017).

The flower of *Rosa damascena* Mill. is an excellent source of various bio-active compounds such as terpenes, flavonoids, glycosides and anthocyanins among which the major constituents are citronellol, geraniol, nerol, quercetin, kaempferol, myercene, gallic acid, linalool etc. (Davoodi *et al*., 2017). This study is designed to investigate hematopoietic i.e. erythropoeitic, leucopoietic and thrombopoeitic potentials of water distilled *Rosa damascena* Mill. flowers (WDRD) in animal model.

MATERIALS AND METHODS

Water distillation of Rosa damascena Mill. flowers

Water distillation was performed to obtain water distilled *Rosa damascena* Mill. flower (WDRD). Fresh flowers of *Rosa damascena* Mill. were purchased from local nursery which were identified and authenticated by Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi [Voucher no: RDF-01-16/17]. Petals were separated from flower and allowed to dry at room temperature. The distillation apparatus comprises of a stainless steel tank, a cohobation column, a condenser and a receiver. Dried petals with distilled water was added in the distillation apparatus in the ratio of 1:2. 2.5 kg of air dried rose petals along with 5 liters of water was added in the distillation apparatus. Air vents were closed after complete removal of air and the apparatus was then operated as a closed system to distill the rose petals under maintained high temperature and pressure. The vapors were generated in cohobated column which were then condensed with circulating chilled water in a condenser and finally received in the receiver. The process of distillation was completed after collection of 1250 ml of distillate. The water distillate of *Rosa damascena* Mill. flower received was of concentration 0.5gm/ml (Babu *et al*., 2002; Osama & Ikram, 2018).

Selection of experimental animals

Male albino rabbits weighing between 1500 to 2000 grams were selected for this study which were purchased

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from local animal supplier. Rabbits were kept in animal house of Pharmacology Department in University of Karachi for 8 to 10 days for the purpose of conditioning and acclimatization. Throughout the entire study period, the animals were kept in normal room temperature of 23 ± 3ºC and humidity 52 to 62% under 12 hour light (07:15 a.m. to 07:15 p.m.) and dark (07:15 p.m. to 07:15 a.m.) cycle with 24/7 free access to standard food and pure water. Rabbits were handled as per the specifications of National Research Council (NRC) (National Research Council, 1997). This study was conducted after the approval of BASR (Board of Advanced Studies and Research), University of Karachi [BASR/No./03460/ Pharm.Resol.No. 10(P) 04].

Animal grouping and dosing protocol

3 groups of 10 rabbits were set for hematological screening. Group I was control and was given 1ml distilled water, Group II and III were test groups and were given water distilled *Rosa damascena* Mill. flower (WDRD) in the doses of 250mg/kg and 500mg/kg respectively (Hajhashemi *et al*., 2010). The dosing was done by oral route for 60 consecutive days and blood was withdrawn for hematological testing on day 31st and day 61st to observe the effect of WDRD on blood cells.

Hematological investigation

For hematological testing including Hgb (hemoglobin), RBCs (red blood cells), WBCs (white blood cells), and PLT (platelet count), approximately 3ml of blood was taken in Bio-Vac EDTA-K_3 tubes. These hematological tests were performed on Automatic Humacount-Plus (HUMAN, Germany) using standard kits which were purchased from HUMAN.

STATISTICAL ANALYSIS

Statistical analysis and data entry was performed on Statistical Package for Social Sciences (SPSS) software version 22. Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. All p-values less than 0.05 were considered significant where $^{*yz}p<0.05$, $^{**yyzz}p<0.01$ and $^{***yyyzzz}p<0.001$ represents level of significance i.e. significant, very significant and highly significant difference in comparison to control, 250mg/kg dose group and day 31st of dosing respectively.

RESULTS

Table 1, 2, 3 and 4 represents the effect of water distilled *Rosa damascena* Mill. flowers (WDRD) on hemoglobin (Hgb), Red Blood Cells (RBCs), White Blood Cells (WBCs) and Platelets (PLT) respectively.

ANOVA (one-way) followed by multiple comparison (Tukey's) test showed that in comparison to control group, both doses i.e. 250 mg/kg and 500mg/kg of WDRD significantly improved the hemoglobin (Hgb), Red Blood Cells (RBCs), White Blood Cells (WBCs) and Platelets (PLT) after 30 and 60 days of dosing.

Throughout the entire study period, both doses of WDRD showed similar effects on hemoglobin (Hgb), Red Blood Cells (RBCs), White Blood Cells (WBCs) and Platelets (PLT) and no significant difference was observed between them after 30 and 60 days of dosing.

Table 1: Effect of WDRD on Hgb (Hemoglobin)

Groups	Hemoglobin (Hgb) g/dL	
	Day 31st	Day 61st
	X ± S.D	X ± S.D
Control I	8.33±0.65	8.47±0.66
Test II (250mg/kg)	10.9±0.42***	10.65±0.64***
Test III (500mg/kg)	10.91±0.5***	11±0.94***

Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. P value $^{***}p<0.001$ represents high level of significance in comparison to control

Table 2: Effect of WDRD on RBCs (Red Blood Cells)

Groups	RBCs (Red Blood Cells) (million/µL)	
	Day 31st	Day 61st
	X ± S.D	X ± S.D
Control I	3.53±0.27	3.32±0.22
Test II (250mg/kg)	5.25±0.24***	5.69±0.42^{***z}
Test III (500mg/kg)	5.45±0.39***	5.83±0.48***

Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. P value $^{***}p<0.001$ represents high level of significance in comparison to control, whereas $^{z}p<0.05$ represents significant difference in comparison to day 31.

In comparison to day 30th of dosing, WDRD in the dose of 250 mg/kg significantly improved the Red Blood Cells (RBCs) and both doses of WDRD significantly improved the Platelet count after 60 days of dosing.

DISCUSSION

Anemia is characterized by decrease RBCs (red blood cells) count followed by decrease in concentration of hemoglobin as well as altered morphology of red blood cells. Low count of red blood cells and hemoglobin concentration leads to impaired oxygen delivery to the body tissues and cause confusion, fatigue, weakness and poor working performance (Kassebaum *et al*., 2014). The conventional or allopathic drugs have various disadvantages like high cost and parenteral administration. Today there is an increase demand for new, economical and orally administered erythropoeitic agents to be used as an alternative and supportive therapy for the treatment and management of anemia (Kim *et al*., 2013).

Our findings revealed that at both doses, water distilled *Rosa damascena Mill*. flower (WDRD) markedly improved the Hgb (hemoglobin) concentration and RBCs (red blood cells) count which shows that WDRD possesses a bone marrow stimulatory action (Riaz *et al*., 2013).

The complex protein hemoglobin (Hgb) consists of heme and globin molecules. It is synthesized in the immature red cells. Hemoglobin is responsible to carry and deliver oxygen and to remove carbon dioxide from the body (George-Gay & Parker, 2003). Low hemoglobin concentration is indicative of anemic state (Fraser & Tyliard, 2008). Water distilled *Rosa damascena* Mill. flower (WDRD) at both doses improves hemoglobin concentration throughout the entire study. Erythrocytes/ Red blood cells are responsible to deliver O_2 from lungs to all body cells and to carry back CO_2 from the body cells to the lungs for removal. Red blood cell count indicates the O_2 carrying capacity of the blood (George-Gay & Parker, 2003). WDRD at both doses markedly improved the red blood cells count throughout the entire study.

Erythropoeitic potential of WDRD might be due to its anti-oxidant action. Flavonoids rich nature is responsible for anti-oxidant action of WDRD (Yassa *et al*., 2015). High oxidative stress and free radicles concentration causes hemolysis. Natural anti-oxidants have the potential to scavenge free radicles and thus can prevent hemolysis (Fibach & Rachmilewitz, 2008). Administration of anti-oxidant rich supplementation leads to increased hematopoiesis. Natural flavonoids also contribute in hematopoiesis (Ekpenyong *et al*., 2011). Recently few researches have recommended that administration of iron supplements along with natural flavonoids such as quercetin improves the hematological profile, increase iron tissue availability in the spleen and ferritin expression in anemic states (Mazhar *et al*., 2017). Hence, these findings show beneficial effects of WDRD which might be helpful in treating anemias.

Leucocytes or white blood cells (WBCs) constitute the major part of body's immune system. They play a

Table 3: Effect of WDRD on WBCs (White Blood Cells)

Groups	WBCs (White Blood Cells) ($10^{\wedge 9}$/L)	
	Day 31st	Day 61st
	X ± S.D	X ± S.D
Control I	4.46±0.32	4.27±0.29
Test II (250mg/kg)	5.65±1.5*	5.89±0.71***
Test III (500mg/kg)	5.91±0.76**	5.97±0.85***

Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. *p<0.05, **p<0.01 and ***p<0.001 represents level of significance i.e. significant, very significant and highly significant difference in comparison to control

Table 4: Effect of WDRD on Platelets Count

Groups	Platelet count ($10^{\wedge 9}$/L)	
	Day 31st	Day 61st
	X ± S.D	X ± S.D
Control I	290.12±44.72	281.56±35.9
Test II (250mg/kg)	468.01±101.19***	567.7±87.01***z
Test III (500mg/kg)	464.21±109.04**	560±42.47***z

Values were presented as Mean ± Standard Deviation (S.D). One-way ANOVA followed by multiple comparison post hoc Tukey's test was performed for statistical calculations. P value **p<0.01, ***p<0.001 represents very and highly significant difference in comparison to control, whereas zp<0.05 represents significant difference between day 31 and 61.

significant role in body's immune system by fighting against foreign invasions and infectious diseases. Doctors and Physicians generally focus on total leucocyte or white blood cell count as a tool to monitor the progress of healing in patients (Riaz *et al*., 2013). Interest in herbal and plant derived products has significantly increased due to their ability to stimulate cellular components of immune system (Haffor, 2010). The effect of WDRD on white blood cells count was also evaluated in this research.

The findings of this study revealed that both doses of WDRD, improved white blood cells count drastically. This leucopoietic activity was more pronounced after 60 days of dosing in comparison to 30 days of dosing. This effect of WDRD might be due to its ingredients eugenol and citronellol. Studies conducted by Kumar *et al* (2012). and Elelaimy *et al* (2012) reported leucopoietic action of eugenol. Zhuang *et al* (2009). Reported immuno-modulatory and wound healing properties of citronellol. Presence of both eugenol and citronellol in water distillate of *Rosa damascena* Mill. flower is reported by Verma *et al* (2011). Hence these findings are indicative of protective action of WDRD against immuno-suppression (Riaz *et al*., 2013).

Thrombocytes or Platelets play a major role in hemostasis and blood coagulation. Usually increase platelet count causes thrombosis and decrease platelet count leads to bleeding (Riaz *et al*., 2013). Thrombocytopenia which is a condition characterized by decrease platelet count of less than 50,000/mm^3 is a frequent complication of many infectious diseases affecting both adult and pediatric population. Numerous factors are responsible for causing thrombocytopenia including increased platelet clearance by mono-nuclear phagocyte system, disseminated intra-vascular coagulation (DIC), direct damage to platelets by virus and/or bacteria, bone marrow suppression or immune mediated thrombocytopenia etc. Bleeding is one of the major complications associated with thrombocytopenia (Burns *et al*., 1991). The conventional agents available to treat thrombocytopenia are associated with certain side effects and are very costly (Gammulle *et al*., 2012). In this study the effect of WDRD on platelet count was also evaluated.

The findings of this study revealed that both doses of WDRD significantly improved the platelet count throughout the entire study and this thrombopoeitic effect was more pronounced after 60 days of dosing. This high platelet count was within the normal acceptable range and may not cause any thrombotic event. The thrombopoeitic potential of WDRD might be due to its constituent quercetin and kaempferol. According to Atik & Rahmadi (2018) quercetin stimulates cell differentiation of megakaryotes and promotes thrombopoiesis. According to Tahir *et al* (2014) plant flavonoids such as kaempferol quercetin etc. have stimulant effect on blood cell production. Presence of both quercetin and kaempferol in water distillate of *Rosa damascena* Mill. flower is reported by Solimine *et al* (2016).

CONCLUSION

In the light of above discussed evidences it can be concluded that water distilled *Rosa damascena* flowers have beneficial and stimulatory effects on blood cell count and possess marked hematopoietic i.e. erythropoeitic, leucopoietic and thrombopoeitic potential which might be helpful in treating anemic, leucopenic and thrombopenic conditions. However more detailed research is required in future to explore its effect in disease associated cytopenic conditions.

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