Biotechnological
Communication

Biosc.Biotech.Res.Comm. Vol 13 (2) April-May-June 2020 Pp-601-609

BBRC
Bioscience Biotechnology
Research Communications

Phytomedicinal Potential of Ethanolic Extracts of Some Trees and Herbs from Thal Desert: *In vitro* Assessment of Plant Antioxidants Effects on Human Haematological Attributes

Iqra Anwer[1], Ghulam Yasin[1],* Mubasharah Sabir[1], Iqra Majeed[1], Farah Akmal[1] and Ikram ul Haq[2],

[1]*Department of Botany, Bahauddin Zakariya University, Multan.Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering (IBGE) University of Sindh, Jamshoro. Pakistan*

ABSTRACT

Secondary metabolites synthesized in desert plants have medicinal properties on one hand and toxicity nature on the other hand. The use of such plants as food or drugs by human being needs to explore their toxic or friendly nature. The effects of secondary metabolites on hematological parameters can provide an insight for finding out their nature. Ethanolic extracts sourced from a variety of plant species are considered to have the potential for inducing changes in human haemotological indices which, when altered, can have pivotal role in determining human health conditions. Aiming to explore this, the ethanolic extracts of some plants from Thal desert of Pakistan were used to determine their effects on some human hematological attributes like counts for granulocyte; leukocytet; eosinophils; monocyte; lymphocyte; granuloytes and lymphocytes. Haemoglobin (Hb), Red Blood Cell (RBC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Packed Cell Volume (PCV), Hematocrit (HCT) and Red Cell Distribution (RDW) were also studied. Eethanolic extracts of some trees and herbs collected from Thal desert were mixed with human blood in 1:4 ratio and tested for complete blood count tests (CBC) using Automated Hematology Analyzer machine. The blood without addition of extract was treated as control. Data were statistically analyzed by using one way ANOVA (Analysis Of Variance). The level of statistical significance was $P < 0.05$. Mean values were differentiated by Duncan's multiple range tests. Among the studied blood characteristics, eosinophils perctage and count, granulocytes percentage, RBC, MCHC, MPV were decreased by all plant extracts. RDW were decreased by extracts of *Tamarix aphylla* stem *Capparis aphylla* stem and root, Lymphocytes were lowered by *Orobanche* stem extract. All other extracts increased the components of blood as comared to control except platelets decline by *Capparis decidua* stem extract.

KEY WORDS: PHYTOMEDICINAL POTENTIAL, ETHANOLIC EXTRACTS,THAL DESERT, IN VITRO, ANTIOXIDANTS, HUMAN BLOOD.

ARTICLE INFORMATION

*Corresponding Author: yasingmn_bzu@yahoo.com
Received 19th April 2020 Accepted after revision 14th June 2020
Print ISSN: 0974-6455 Online ISSN: 2321-4007 CODEN: BBRCBA

Thomson Reuters ISI Web of Science Clarivate Analytics USA and Crossref Indexed Journal

NAAS Journal Score 2020 (4.31) SJIF: 2020 (7.728)

Online Contents Available at: http//www.bbrc.in/
DOI: 10.21786/bbrc/13.2/36

INTRODUCTION

In whole history of humanity, in his struggle against diseases, man has seeked help from plants which are the sources of biologically-active nature of health-promoting constituents. The use of plants in treating ailments is too old to date back 1550 BC (Petrovska, 2012, Kumar et al. 2019). Almost every medical systems, may it be Traditional Medicine, Ayurvedic medicine, Kampo medicine, European medicine, is based on valuable plant derived medicinal substances (Lu et al. 2016). Currently, medicine is faced with a growing demand for a wide range of biologically-active compounds of natural origin that can demonstrate preventive or therapeutic. A large proportion of the phytochemicals used for effects against causes of death, such as cardiovascular disease, cancer, diabetes or respiratory disease are secondary metabolites. These compounds have many different functions in plant itself such as protecting plants from pathogens (Zaynab et al. 2018: Bednarek et al. 2018), herbivores (Huang et al.2019; Huber et al. 2016) and ultraviolet light (Köhler et al. 2017; Takshak and Agrawal 2019).

They also provide color and fragrances to facilitate seed dispersal and pollination by animals. These play an important role as signals and regulatory molecules in primary metabolism. In modern medicine, plant secondary metabolites play a vital role. Secondary metabolites have antioxidant (Zlatic et al. 2019; Gonçalves 2019), antibacterial (Barbieri et al. 2017), anti-inflammatory (Bernstein et al. 2018, Flores-Sánchez et al. 2019), antifungal (Reichling 2010Lagrouh et al. 2017), hepatoprotective (Pereira et al. 2016) and neurological (Epifano et al.2008) effects. The increasing uses of these plant secondary metabolites demands the use of new biotechnological tools to create new and productive transgenic plant cultures. (Kowalczyk etal. 2020).

Plants products have been used as medicine since long time owing to their availability, no side effects and easy usage. In developing countries including like Pakistan, even today, the practice of medicine relies on plants and their products. Plant extract based medicine is culturally well accepted by peoples of urban and rural areas. Many plants contain number of secondary metabolites such as saponins, phenols, necessary oils, and many other phytochemicals. These metabolites have medicinal properties on one hand (Aggarwal et al. 2003) and have been reported to be toxic on the other hand (Jennifer et al. 2005). These secondary metabolites have been well documented to be present in plants of the adverse environmental conditions such as deserts and act as antioxidants for scavenging reactive oxygen species (ROS).

Deserts are arid and semi-arid lands which comprise approximately one third of the world's land surface and are under constant threat of drought (Wickens, 1998). In arid regions, drought occurs regularly and annual evaporation frequently more than total amount of annual precipitation. Due to these environmental conditions, the vegetation of the desert comprises xerophytic species which are adapted to these various environmental stresses, like extreme aridity, high salinity, high temperature and low nutrient availability (Naz et al, 2010). Water deficiency also affects many aspects of the plant from cell function to mechanistic properties stress and it will have great importance in worldwide both economically and environmentally (Lambers, 1998 and Rachmilevitch et al. 2006) Plants are adapted to these adverse environmental conditions by synthesizing secondary metabolites. Some of the plant secondary metabolites show negative effects on health, survival and behaviour of consumer especially herbivores because of the harmful toxins (Jennifer et al. 2005).

Concentration of these metabolites in plants and nature of their toxicity for consumer vary with stress severity and type (Close and McArthur, 2002). Some common and the most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Moreover, a number of medicinal plants containing flavonoids and alkaloids are used in natural medicine and are known to contain important therapeutic agents. Their identification needs to be interpreted in the light of traditional use and preparation of plant drugs (Taylor et al. 2001). Plants need to be well diagnosed for their medicinal potential, antioxidant status and toxicity nature of secondary metabolites prior to its use for human health. The use of herbal preparations, without any proper scientific studies on their safety, can raise concerns on their toxicity (Saad et al. 2006).

Furthermore, blood parameters reveal the health status of an individual. This is because blood plays a vital role in physiological, nutritional and pathological status of organism. Of these, Physiological parameter could be a valuable means of diagnosing health of an individual (Ganong, 2005). Hence, the exploration of various blood parameters might be useful criteria that can be used to assess the toxic or medicinal potentials of plant extracts (Sunmonu, 2010). Effects of herbal extract on animals are commonly used to deduce potential health risk for humans indirectly (Ashafa, 2011). However, direct evaluation of in vitro toxicity effects on human hematological parameters, can have more predictive value for human health. If no ethnomedicinal knowledge available, plant can be selected randomly and screened for medicinal constituents derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. After a careful experimental testing for its toxicity or medicinal potential, a plant can be used for medicinal purposes.

MATERIAL AND METHODS

Our experiment is aimed to explore the toxic or medicinal nature of ethanol soluble secondary metabolites (Hemwimon et al. 2007) of desert plants. Toxic or medicinal effects of herbal extract are commonly used in animals to deduce their potential health effects on humans indirectly (Ashafa, 2011). However, in vitro evaluation of toxicity effects on human hematological parameters directly, can have predictive value for human

health. To assess the ethno-medicinal or toxicological validation of plant secondary metabolites, the desert plants were selected. The selection of desert plants is based on arid environmental conditions which enforce plants to synthesize secondary metabolites for adaptation to stressful environment (Naz et al. 2010). Desert plants have traditional utilization in the treatment of some diseases and free radicals related disorders by local peoples with no proper documentation of their side effects (Taylor et al. 2001). Moreover, the choice of in vitro utilization of human blood is based on the role blood plays in determining the physiological, nutritional and pathological status of organism. Among these, physiological parameters are dependent on blood attributes and could be a valuable means of diagnosing a disease (Ganong, 2005). Hence, the investigation of various hematological parameters might be a useful index that can be employed to assess the toxic or useful potentials of plant extracts containing secondary metabolites (Sunmonu and Oloyede, 2010).

Experimental design: Using ethanol as solvent, plant extract was prepared (Kinuthia et al 2014, and Saha, 2008). Human blood from a healthy volunteer was analysed for haemotological indices after mixing with plant extract (Ughachukwu et al. 2013 and Sayeed et al. 2014). Three samples of each extract were pooled to get means to reduce the error. A comparison with normal blood characteristics was statistically calculated.

Field survey and trees sample collections: Thal desert is located in Punjab provice of Pakistan lying between Indus and Jehlum rivers. It extends over an area of 23000 square kilometers between 70.8°- 72°E longitude and 30° – 32.5° N latitude. (Mares, 2017). In a preliminary survey of Thal desert, meetings with local peoples were arranged to know the geographical area and local plant names. Intact specimens were collected and herborized from the study area for each new plant species present and mentioned by local people, Herborized specimens were identified specialists, by matching them with the labelled herbarium exsiccates lying in the departmental herbarium (Dr.Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakarya University, Multan Pakistan and/or the literature (Ali,1993). Data and specimens were collected according to an appropriate methodology (Jain, 1995; Khan 1993) keeping uniformity among age of plants, size of plants and size of sand dunes. Further processing of collected specimens was carried out in laboratory of the department.

Table 1. In vitro effect of ethanolic extract of some trees and herbs of Thal on human hematology[values represent mean±standard daviation; n=3]

Name of species	leukocyte count (10×3/u L)	Granulocyte (%)	Lymphocyte (%)	Monocyte (%)	Eosinophils (%)	Granulocyte (10×3/L)
Normal blood	5.63 ± 0.05 k	0.13 ± 0.05 o	47.93 ± 0.05 i	1.96 ± 0.05 m	2.76 ± 0.05 a	0 ± 0 d
Tamarix aphylla (r)	7.26 ± 0.65 j (+28.95)	32.53±1.05 e (+24923.08)	58.53 ± 1.106 bc (+22.11)	6.43 ± 1.105 k (+228.06)	0.20± 0.1 cd (-92.75)	2.56 ± 1.22 c (-256)
Capparis decidua (s)	10.13 ± 0.60 efg (+79.92)	23.13± 1.305 i (+17692.31)	55 ± 0.9bcd (+14.75)	20.56 ± 0.95 fg (+948)	0.03 ± 0.05 e (-98.91)	2.5 ± 1.25 c (-250)
Capparis decidua (r)	8.20± 0.55 hij (+45.64)	21.43± 0.83 ij (+16384.62)	54.43± 1.02 bcd (+13.56)	19.6± 0.75 g (+900)	0.03 ± 0.05 e (-98.91)	2.56± 1.22 c (-256)
Fagonia arabica (s)	9.20± 0.55 gh (+63.41)	31.36 ± 0.8 ef (+24023.08)	53.1 ± 0.95 bcde (+10.85)	11.53 ± 1.05 j (+488.26)	0.40± 0.1 b (-85.71)	2.9 ± 1 c (-290)
Orobanche aegyptica (f)	16.56 ± 0.87 c (+194.13)	31.83 ± 1.05 ef (+24384.62)	54.5 ± 0.91 bcd (+13.77)	13.5 ± 1.1 i (+588.77)	0 ± 0 e (-100)	5.46 ± 0.96 b (-546)
Orobanche aegyptica (s)	17.13 ± 1.15 bc (+204.26)	43.3 ± 1.6c (+33207.69)	40.3 ± 0.90 fgh (-15.86)	16.56 ± 1.2 h (+744.89)	0 ± 0 e (-100)	7.6 ± 1.08 a (-760)
Citrullus colocynthis (r)	10.66 ± 0.90 ef (+89.34)	20.76 ± 1.10 j (+15869.23)	57.36 ± 0.80 bc (+11.32)	19.36 ± 0.86 g (+887.75)	0.3 ± 0.1 bc (-89.28)	2.66 ± 1.07 c (-266)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

Crude ethanolic extract preparation:The collected specimens were first washed with water and later on with 2% ethanol to remove dust and other surface contaminants, dried at room temperature, and were grounded to fine powder using pestle and mortar. Following the procedure adopted by Afolayan et al. (2010), crude ethanolic extract was prepared from finely grounded 100.0 g plant material in 200ml ethanol by shaking at room temperature for 3h. The ethanol used was of highest purity. The extract was filtered and residue was re-processed for extraction. Solvent was evaporated by rotory evaporator at and material was stored at -4°C.

Blood sampling and in vitro analysis: After ensuring the confidentiality and anonymity to a blood donor and approval from local ethical committee, human blood was obtained from a healthy volunteer of 25 years age having O+ blood group. The volunteer was selected after a questionnaire of not taking any medications or addictive substances (including tobacco, alcohol and aspirin or any other anti-platelet drugs) and keeping a balanced diet (meat and vegetables); using no antioxidant supplementation. By adding ethanol, plant extract was diluted up to 5ml. After consulting literature, the ratio of mixing blood to plant extract was determined by trial method to find appropriate dose when no coagulation occurred. Finally, plant extract was added into 4ml blood (1:4) and was shaked smoothly. Blood sample without addition of extract was considered as control for comparison. Complete blood count tests (CBC) by using Automated Hematology Analyzer machine was performed for hematological indices.

Statistical analysis of data: Data obtained for blood test were analyzed by using one way ANOVA (Analysis Of Variance) at 5% level of statistical significance. Means were compared by Duncan's multiple range test (Duncan, 1955).

Table 2. Table In vitro effect of ethanolic extract of some trees and herbs of Thal on human heamatology[values represent mean±standard daviation; n=3]

Name of species	Lymphocyte count (10×3/L)	Monocyte count (10×3/L)	Eosinophils count (10×3/L)	RBC (10×6/ uL)	HGB (g/dL)	HCT (%)
Normal blood	2.68 ± 0.02 j	0.13 ± 0.05g	50.33 ± 0.57 h	5.14 ± 0.005 c	8.33 ±0.05 d	22.73 ± 0.05h
Tamarix aphylla (r)	4.56 ± 1.19 efgh (+70.14)	0.5 ± 0.1fg (+284.61)	2.73 ± 0.90 cde (-94.57)	4.28 ± 0.95 bc (-16.73)	8.66 ± 1.02 bcd (+3.96)	24.66 ± 1.05e (+71.84)
Capparis decidua (s)	5.7 ± 1.15 cde (+112.68)	2.6 ± 1.04bcd (+1900)	1.6 ± 0.2efg (-96.82)	3.7 ± 1.113 bc (-28.01)	8.63 ± 1.05 bcd (+3.60)	20.83 ± 1.006h (+54.993)
Capparis decidua (r)	5.73 ± 1.06 cde (+113.80)	2.63± 1.15bcd (+1923.07)	1.46 ± 0.15 fg (-97.09)	4.45± 1.19 bc (-13.42)	8.43 ± 1.00 bcd (+1.20)	24.7± 1.15e (+72.01)
Fagonia arabica (s)	4.8 ± 1.1 defgh (+79.10)	1 ± 1 efg (+669.23)	1.46 ± 0.15 fg (-97.09)	4.56 ± 0.92 bc (-11.45)	8.63±1.02bcd (+3.60)	27.63 ± 1.05c (+21.7)
Orobanche aegyptica (f)	9.4 ± 0.62 a (+250.74)	2.66 ± 1.00bcd (+1946.15)	4.53 ± 1.11 b (-90.99)	3.78 ± 1.095 bc (-26.60)	8.36±1.00bcd (+0.36)	25 ± 1 de (+9.98)
Orobanche) aegyptica (s	7.63 ± 1.13 ab (+184.70)	3 ± 1 bc (+2207.69)	0 ± 0 h (-100)	4.28 ± 0.92 bc (-16.89)	9.46±1.15abc (+13.56)	24.7 ± 1.05e (+8.66)
Citrullus colocynthis (r)	6.5 ± 0.79 bcde (+142.53)	2.53 ± 1.1 bcd (+1846.15)	0± 0 h (-100)	4.38 ± 1.30 bc (-14.95)	9.63±1.12abc (+15.60)	24.63 ± 1.10 e (+8.35)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

RESULTS AND DISCUSSION

Tamarix aphylla root: Ethanolic extract of *Tamarix aphylla* root substantially altered blood parameters. The extract had increased Leukocyte (28.95%), granulocyte count (24923.08%), lymphocyte count (22.11%), Monocyte count (228.06%), lymphocyte (70.14%), Monocyte (284.61%), HGB (3.60%), HCT (71.84%), MCV (32.33%), MCH (40.91%), RDW (12.37%) and platelets (42.96%) respectively. Ethanolic extract had affected Eosinophils count, granulocyte, RBC, Eosinophils, MCHC, and MPV with different degrees there by lowering values from control as Eosinophils count (98.91%), granulocyte (256%), RBC (16.73%), Eosinophils (94.57%), MCHC (7.45%), and MPV (28.86%) .

***Capparis decidua stem*:** Ethanolic extract of *Capparis* decidua stem proved its significant influence . Mean values revealed that an increse was noted for leukocyte (79.92%), granulocyte count (17692.31%), lymphocyte count (14.75%), Monocyte count (948%), lymphocyte (112.68%), Monocyte (1900%), HGB (3.60%), HCT (54.993%), MCV (21.75%), MCH (23.99%), MCHC (16.29%) and RDW (11.34%). An exception in this correlation was found MPV, RBC, platelets, Eosinophils, granulocyte, Eosinophils count parameters. Blood parameters MPV (28.42%), platelets (25.48%), RBC (28.01%), Eosinophils (96.82%), Granulocyte (250%) and Eosinophils count (98.91%) revealed a significant decreased from control.

***Capparis decidua* root:** Ethanolic extract of *Capparis* decidua root change in blood parameters. The most marked increase was in leukocyte (45.64%), Granulocyte count (16384.62%), lymphocyte count (13.56%), Monocyte count (900%), lymphocyte (113.80%), Monocyte (1923.07%), HGB (1.20%), HCT (72.01%), MCV (31.42%), MCH (40.73%) and RDW (18.63%). The application of extract seems to decreased significantly Eosinophils count (98.91%), Granulocyte (256%), Eosinophils count (97.09%), RBC (13.42%), MCHC (8.81%) and MPV (33.62%).

***Fogonia arabica* stem:** Different sensitivity range was found in response of blood parameters treated with ethanolic extract of Fogonia arabica stem. The application of extract seems in enhancing the leukocyte (63.41%), granulocyte count (24023.08%), lymphocyte count (15.68%), Monocyte count (488.26%), lymphocyte count (10.85%), lymphocyte (79.10%), Monocyte (669.23%), HGB (3.60%) HCT (21.7%), MCV (31.92%), MCH (29.57%), RDW (49.34%) and platelets (18.69%) from control. But the extract decreased MPV, MCHC, granulocyte, Eosinophils, Eosinophils count and RBC parameters. The maximum decrease was for MPV (21.21%), MCHC (11.39%), granulocyte (290%), Eosinophils count (85.71%), RBC (11.45%) and Eosinophils (97.09%) from control.

***Orobanche aegyptica* flower:** Eethanolic extract of *Orobanki aegyptica* flower. increased the leukocytes (194.13%), granulocytes count (24023.08%), lymphocyte count (13.77%), Monocytes count (588.77%), lymphocyte (250.74%), Monocytes (1946.15%), HGB (0.36%), HCT (9.98%), MCV (48.14%), MCH (9.29%), RDW (90.21%) and platelets (82.55%) from control. But the extract decreased the values for MPV, RBC, MCHC, Eosinophils count, Eosinophils and granulocytes parameters. The maximum decrease was for MPV (6.78%), MCHC (9.39%), granulocyte (290%), Eosinophils count (100%), RBC (26.60%) and Eosinophils (90.99%) from control.

***Orobanche aegyptica* stem:** The extract of *Orobanki aegyptica* stem changed blood parameter A significant increase in leukocytes (204.26%), granulocytes count (33207.69%), Monocyte count (744.89%), lymphocyte (184.70%), Monocyte (2207.69%), HGB (13.56%), HCT (8.66%), MCV (37.25%), MCH (39.05%), MCHC (2.45%), RDW (74.76%) and platelets (100.26%) was noted. Ethanolic extract rdecreased MPV, Eosinophils count granulocyte lymphocyte count parameters by values of MPV (23.52%), Eosinophils count (100%), granulocytes (760%) and lymphocyte count (15.86%).

***Citrollus colocynthis* root:** *Citrollus colocynthus* root Ethanolic extract also changed the blood parameters. The significant increase was in leukocyte count (89.34%), granulocyte count (15869.23%), lymphocyte count (11.32%), monocyte count (887.75%), lymphocyte (142.53%), HGB (15.60%), HCT (8.35%), MCV (40.13%), MCHC (1.17%) and RDW (94.54%). The application of extract decreased significantly MPV (44.73%), granulocyte (266%), Eosinophils count (89.28%), RBC (14.95%), Eosinophils (100%) from control.

The hematological parameters assessment acts as diagnostic criteria for effects of any foreign compounds on the blood components (Ashafa et al. 2012). Addition of chemical compounds at toxic level changes the blood parameters indicating hematological disorders like anemia which is characterized by low hemoglobin content (Price and Schrier 2008)); decline in platelet results in lymphoma and myeloma (Izak and Bussel 2014; Bradbury and Murray 2013). Blood parameters are used for diagnosing the physiological status of an organism (Pankaj and Varma 2013). Low levels of hemoglobin and RBC is due to iron deficiency or blood cell destruction which to anemia (Junqueira et al. 2006).

The increased levels of free hemoglobin in blood (hemoglobinemia) might be as a result of massive hemolysis Ugwu et al. 2013). Hematocrit (PCV) (called Packed Cell Volume (PCV) is clinically used to signal known or suspected anemia (Wintrobe and Greer 2009). Mean cell/corpuscular volume (MCV) is volume or size of a red blood cell. High MCV mean larger RBC size). Low MCV is due to iron deficiency (Aslinia et al. 2006). Mean corpuscular hemoglobin (MCH) is average amount of hemoglobin in single red blood cell. Invrease in MCH is due to anemias (Kasper et al. 2005). Mean corpuscular hemoglobin concentration (MCHC) is also average concentration of hemoglobin inside a single red blood cell. A low MCHC is iron deficiency orindication of abnormal hemoglobin synthesis MCV is size of red blood cells while MCH and MCHC are for concentration of hemoglobin. White blood cells (WBC) count and its indices play a vital role in immune function. A high number of eosinophils are due to a variety of disorders (Wintrobe and Greer 2009).

Our results revealed a diversified action of ethanolic extracts sourced from different plants parts on various hematological attributes. The results contradict to the findings of Lohar et al. (2009) while are in accordance to the findings of Straus, 1998 regarding effects on RBC and Hb concentration (MCH, MCHC). It has been reported that plants extracts vary in their actions and are often non-specific in their actions (Treasure, 2000). Many plants have been known to produce biologically active substances which are related to their special flavours, taste and antioxidant status. Among the phenolic compounds, antioxidants and secondary metabolites, the most abundant are natural antioxidants (Fiorentino et al. 2006). Antioxidants can act as cell saviors, as reducing agents, free radical scavengers, singlet oxygen quenchers or hydrogen donors (Fattouch et al. 2007).

Antioxidants are synthesized in plants under environmental stresses to neutralize the reactive oxygen species (ROS). Damage to cells and biomolecules caused by reactive oxygen/nitrogen species (ROS/RNS) is nullified by antioxidant (Fiorentino et al. 2006).The

concentration ratio of antioxidant to ROS determines not only the potential of plants to withstand adverse environmental conditions but also when present in plant extract or its products, these might play important role against the hemolytic activity of ROS by stabilizing blood cells and molecules or by their direct action on ROS. The plant organic extracts have high amount of antioxidants such as flavonoids and phenolic. Flavonoids are especially important for protection against human diseases. (Tiwari, 2001).

Erythrocytes are considered as major target of free radicals (ROS) due to the presence of high concentrations of polyunsaturated fatty acids in cell membrane (Ebrahimzadeh et al. 2009). ROS may cause oxidative damage to the erythrocyte membrane due to hemolytic activity. Red Blood Cells (Erythrocytes) are the most abundant cells in the human blood. Medicines can have more effect on Erythrocytes than any other blood cells (Hamidi and Tajerzadeh, 2003). Results of present studies revealed that ethanolic extracts of desert plants specimens reduced the RBC. The hemolysis of red blood cells by ROS, produced in desert plants parallel to stress, damages the cell membrane with release of hemoglobin from these cells. All these factors, in union, cause deterioration of cell membrane, which may, perhaps, be the key step for lysis of cell.

Results revealed that ethanolic extracts of desert plant specimens decreased MCHC and increased MCH and MCV (Table.1). The MCHC and MCH are indices of haemoglobin concentration in blood and in its each cell respectively (Wickramasingh, 1991). Mean cell volume (MCV) is the volume of red cells. An increase in MCV accompanied with a decrease in MCHC could accounts for the reduction in osmotic fragility of cell membrane (Olaleye, 1999). MCH, MCV and MCHC relates to individual red blood cells while Hb and RBC are concerned with the total numbers of red blood cells (Ashafa, 2011). The MCV determines the size of the RBCs. RBCs of normal size are termed normocytic. When the MCV is high, the RBCs will be larger and are termed as macrocytic. When the MCV is below normal, the RBCs are smaller which are called as microcytic. These categories of size are used to classify anemias. A significant reduction in MCV, if observed, accounts for interference in iron uptake by hemoglobin. Furthermore, it is reported that decrease in the blood cells may be due to the increased glycosylation (non enzymatic) of membrane proteins which can cause hyperglycemia.

Table 3. In vitro effect of ethanolic extract of some trees and herbs of Thal on human heamatology[values represent mean±standard daviation; n=3]

Name of species	Lymphocyte count (10×3/L)	Monocyte count (10×3/L)	Eosinophils count (10×3/L)	RBC (10×6/ uL)	HGB (g/dL)	HCT (%)
Normal blood	2.68 ± 0.02 j	0.13 ± 0.05 g	50.33 ± 0.57 h	5.14 ± 0.005 c	8.33 ±0.05 d	22.73 ± 0.05 h
Tamarix aphylla (r)	4.56 ± 1.19 efgh (+70.14)	0.5 ± 0.1fg (+284.61)	2.73 ± 0.90 cde (-94.57)	4.28 ± 0.95 bc (-16.73)	8.66 ± 1.02 bcd (+3.96)	24.66 ± 1.05e (+71.84)
Capparis decidua (s)	5.7 ± 1.15 cde (+112.68)	2.6 ± 1.04 bcd (+1900)	1.6 ± 0.2efg (-96.82)	3.7 ± 1.113 bc (-28.01)	8.63 ± 1.05 bcd (+3.60)	20.83 ± 1.006 h (+54.993)
Capparis decidua(r)	5.73 ± 1.06 cde (+113.80)	2.63± 1.15 bcd (+1923.07)	1.46 ± 0.15 fg (-97.09)	4.45± 1.19 bc (-13.42)	8.43 ± 1.00 bcd (+1.20)	24.7± 1.15 e (+72.01)
Fogoniaarabica (s)	4.8 ± 1.1 defgh (+79.10)	1 ± 1 efg (+669.23)	1.46 ± 0.15 fg (-97.09)	4.56 ± 0.92 bc (-11.45)	8.63±1.02bcd (+3.60)	27.63 ± 1.05c (+21.7)
Orobankiaegyptica (f)	9.4 ± 0.62 a (+250.74)	2.66 ± 1.00 bcd (+1946.15)	4.53 ± 1.11 b (-90.99)	3.78 ± 1.095 bc (-26.60)	8.36±1.00bcd (+0.36)	25 ± 1 de (+9.98)
Orobankiaegyptica (s)	7.63 ± 1.13 ab (+184.70)	3 ± 1 bc (+2207.69)	0 ± 0 h (-100)	4.28 ± 0.92 bc (-16.89)	9.46±1.15abc (+13.56)	24.7 ± 1.05 e (+8.66)
Citrolluscolocynthus (r)	6.5 ± 0.79 bcde (+142.53)	2.53 ± 1.1 bcd (+1846.15)	0± 0 h (-100)	4.38 ± 1.30 bc (-14.95)	9.63±1.12abc (+15.60)	24.63 ± 1.10 e (+8.35)

Values sharing the different letters represent significance difference in respective row; values in parenthesis represents percentage difference over control group; LSD= least standard deviation; r=root; s=stem; f=flower

Ethanolic extracts of some specimens reduced the haemoglobin concentration while others induced an increase. This change in haemoglobin quantity in blood might be due to Iron deficiency as Iron is a component of heme group in hemoglobin. This Iron deficiency could be due to interference of plant extract biomolecules with iron directly or interference during its biosynthesis metabolism. A failure in production of hemoglobin results in cells smaller than normal cells. This occurs in many diseases, including iron deficiency anemia,

thalassemia, and anemias associated with chronic infection. Iron deficiency might be due to desert plant ROS which cause mobilization of Fe^{2+} by Ca^{2+} via Fenton reaction (Kupier-Goodman and Scott, 1989). The increase in Hb (MCH and MCHC) facilitates oxygen transport to the tissues. An increase in Hb concentration (MCH, MCHC) may be due to the presence of active gradients that stimulate haemopoiesis, or support in availability of iron for haemopoiesis, or agents for chelating iron may be absent or weakly present in the plant extract which change the extent of hemolysis of RBC (Lohar et al, 2009).

An increase or decrease in blood attributes might be owed to free radical scavenging activity of extract (Saha et al. 2008); antiuglycosylation (Nair et.al.,2013); thrombolytic potential (Sayeed et.al. 2014) , anticoagulation by plant extract (Ughachukwu et.al. 2013) or by genotoxicity (Pereira et.al. 2014). Reduction in platelets by ethanolic extracts was observed (Table.1). Blood platelets reduction may be beneficial at some level because platelets decrease the blood viscosity which positively adds to blood pressure and may be beneficial by view of the clinical haematology. Change in Blood platelet might be by their adhesion to collagen and platelet aggregation by ROS species of desert plants (Olas, 2008). Plant antioxidants might change blood platelet by antiplatelet activity (Dutta-Roy, 2002).

Conclusively, based upon the criteria of importance of blood attributes,the extract of Orobanche plant was the most promising in enhancing haemoglobin concentration than other plants. The Capparis decidua stem extract proved to be worst for decreasing platelets of blood. Different blood parameters were influenced to varying extent by these plants antioxidant extracts. Hence, the practical application of the plant antioxidant used should be based upon their careful and extensive study regarding their pharmaceutical or toxicological nature.

REFERENCES

Afolayan, A.J., Aboyade, O.M., Adedapo, A.A., Sofidiya, O.M. (2010). Anti-inflammatory and analgesic activity of the Ethanol extract of *Malva parviflora* Linn. (Malvaceaea) in rats. African J Biotech 9: 1225-1229.

Aggarwal, B.B., Kumar, A., Bharti, A.C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res 22:363-398.

Ali, S.I., Qaiser, M. (Eds) (1993–2011). Flora of Pakistan. Department of Botany, University of Karachi, Pakistan. Nos. 194–218.

Ashafa, A.O., Orekoya, L.O., Yakubu, M.T. (2012). Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. Asian Pac J Trop Biomed 2: 811-817.

Ashafa, A.T., Olunu, O.O. (2011). Toxicological evaluation of ethanolic root extract of *Morinda lucida* (L.) Benth. (Rubiaceae) in male Wistar rats. J Nat Pharm 2:108-114.

Aslinia, F., Mazza, J.J., Yale, S.H. (2006). Megaloblastic anemia and other causes of macrocytosis. Clin Med Res 4: 236-241.

Barbieri, R.. Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S.F., Nabavi, S.M.(2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiol Res 196: 44–68.

Bednarek, P., Schulze-Lefert, P. (2008). Role of Plant Secondary Metabolites at the Host-Pathogen Interface. In Molecular Aspects of Plant Disease Resistance 2018, Parker, J., Ed., Wiley-Blackwell: Hoboken, NJ, USA,34: 220–260.

Bernstein, N., Akram, M., Daniyal, M., Koltai, H., Fridlender, M., Gorelick, J. (2018). Anti-inflammatory Potential of Medicinal Plants: A Source for Therapeutic Secondary Metabolites, 1st ed., Elsevier Inc.: Amsterdam, The Netherlands.

Bradbury, C., Murray, J. (2013). Investigating an incidental finding of thrombocytopenia. BMJ 346: 11.

Close, D.C., McArthur, C. (2002). Rethinking the role of many plant phenolics: protection from photodamage not herbivores? Oikos 99: 166-172.

Duncan, D.B. (1955). Multiple Range and Multiple F-Test. Biometrics 11: 1-42.

Dutta-Roy, A.K. (2002). Dietary components and human platelet activity. Platelets 13:67-75.

Ebrahimzadeh, M.A., Ehsanifar, S., Eslami, B. (2009). *Sambucus ebulus elburensis* fruits: A good source for antioxidants. Phcog Mag 4, 213-218.

Epifano, F., Molinaro, G., Genovese, S., Ngomba, R.T., Nicoletti, F., Curini, M. (2008) Neuroprotective e_ect of prenyloxycoumarins from edible vegetables. Neurosci Lett 443: 57–60.

Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C.I.G., Angioni, A., Dessi, S., Marzouki, N., Cabras, P. (2007). Antimicrobial activity of tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. J Agric Food Chem 55: 963–969.

Fiorentino, A., D'Abrosca, B., Pacifico, S., Mastellone, C., Piscopo, V., Monaco P. (2006). Spectroscopic identification and antioxidant activity of glucosylated carotenoid metabolites from Cydonia vulgaris fruits. J Agric Food Chem 54: 9592–9597.

Flores-Sanchez, K., Cruz-Sosa, F., Zamilpa-Alvarez, A., Nicasio-Torres, P. (2019). Active compounds and anti-inflammatory activity of the methanolic extracts of the leaves and callus from *Tilia americana* var.mexicana propagated plants. Plant Cell Tissue Organ Cult 137: 55–64.

Ganong, W.F. (2005). Review of Medical Physiology (International edition) McGraw Hill Companies Inc, Singapore 25-557.

Gonçalves, S., Mansinhos, I., Rodríguez-Solana, R., Pérez-Santín, E., Coelho, N., Romano, A. (2019). Elicitation improves rosmarinic acid content and antioxidant activity in Thymus lotocephalus shoot cultures. Ind Crops Prod 137: 214–220.

Hamidi, H., Tajerzadeh, H. (2003). Carrier erythrocytes: an overview. Drug Deli 10:9–20.

Haq, I. (2004). Safety of medicinal plants. Pak J Med Res 43:203–210

Hemwimon, S., Pavasant, P., Shotipruk, A. (2007). Microwave- assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia* .Separation and Purification Technology 54: 44-50.

Holan, V., Cechova, K., Zajicova, A., Kossl, J., Hermankova, B., Bohacova, P., Hajkova, M., Krulova, M., voboda, P., Javorkova, E. (2018). The Impact of Morphine on the Characteristics and Function Properties of Human Mesenchymal Stem Cells. Stem Cell Rev Rep 14: 801–811.

Huang, W., Gfeller, V., Erb, M.(2019). Root volatiles in plant–plant interactions II: Root volatiles alter root chemistry and plant–herbivore interactions of neighbouring plants. Plant Cell Environ 42: 1964–1973.

Huber, M., Epping, J., Schulze Gronover, C., Fricke, J., Aziz, Z., Brillatz, T., Swyers, M., Köllner, T.G., Vogel, H., Hammerbacher, A. (2016). A Latex Metabolite Benefits Plant Fitness under Root Herbivore Attack. PLoS Biol 14: 1–27.

Izak, M., Bussel, J.B. (2014). Management of thrombocytopenia. F1000Prime Rep 6: 45.

Jain, S.K. (1995). A Manual of Ethnobotany, 2nd Edition. Scientific Publishers, Jodhpur.

Jennifer, S. S., Heward, E., Dearing, M.D. (2005). Plant secondary metabolites alter the feeding patterns of a mammalian herbivore (*Neotoma lepida*). Oecologia 146: 415–422

Junqueira, L.C., Carneiro, J., Kelley, R.O. (1992). Basic Histology. A Lange medical book. Appleton and Lange pp: 72-125.

Junqueira, L.C., Carneiro, J., Kelley, R.O. (2006). Basic Histology. A Lange Medical Book. Appleton and Lange (7th edn): 72-125.

Kasper, D.L., Braunwald, E., Fauci, A., Hauser, S., Longo, D. (2005). Harrison's principles of internal medicine. 32. Mahmoud AM (2013) Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. EXCLI J 12: 647-657.

Khan, S.S. (1993). Ethnomedicinal studies on plants of Bhopal district of M.P, Ph.D.Thesis, Barkatullah University, Bhopal.

Kinuthia, G.K., Kabiru, E.W., Anjili, C.O., Kigondu, E.M., Ngure, V.N., Ingonga, J.M. Gikonyo, I.K. (2014). Efficacy of crude ethanolic extracts of *Allium sativum* L. and *Moringa stenopetala* (Baker f.) Cufod. Against Leishmania major. Intro J Med Aroma Plants 4: 16-25.

Kohler, H., Contreras, R.A., Pizarro, M., Cortes-Antíquera, R., Zuniga, G.E. (2017). Antioxidant responses induced by UVB radiation in Deschampsia Antarctica Desv. Front Plant Sci 8: 1–10.

Kowalczyk , T., Wieczfinska, J., Skała, E., Sliwinski, T. and Przemysław Sitarek (2020). Transgenesis as a Tool for the Efficient Production of Selected Secondary Metabolites from Plant in Vitro Cultures.Plants. DOI:10.3390/plants9020132

Kumar, A., Kumar, S., Thomas, T.D., Ramchiary, N., Swamy, M.K., Ahmad, I. (2020). Linking Omics Approaches to Medicinal Plants and Human Health. In Natural Bio-Active Compounds, Springer: Berlin/ Heidelberg, Germany, 2019, pp. 31–57. Plants 9:14-21

Kupier-Goodman, T., Scott, P.M. (1989). Risk Assessment of mycotoxin ochratoxin A. Biomedicine and Envir Sci 2:179-248.

Lagrouh, F., Dakka, N., Bakri, Y. (2017). The antifungal activity of Moroccan plants and the mechanism of action of secondary metabolites from plants. Journal de Mycologie Medicale 27: 303–311.

Lohar, P.S., Lohar, M.S., Roychoudhury, S. (2009). Erythropoitic effects of some medicinal plants of India on experimental model. Slovak J Animal Sci 42: 95-98.

Lu, X., Tang, K., Li, P. (2016). Plant metabolic engineering strategies for the production of pharmaceutical terpenoids. Front Plant Sci 7: 1–11.

Mare, MA.(2017) Encyclopedia of deserts.University of Oklohama press, Norman, USA. p: 291.

Nair, S.S., Kavrekar, V., Mishra, A. (2013). Evaluation of *In Vitro* Anti diabetic Activity of Selected Plant Extracts. Int J Pharm Sci 2: 12-19.

Naz, N., Hameed, M., Ahmad, M.S.A., Ashraf, M., Arshad, M. (2010). Is soil salinity one of the major determinants of community structure under arid environments? Commun. Ecol 11: 84-90.

Olaleye, S.B., Iranloye, B.O., Salami, H.A., Elegbe, R.A. (1999). Biosci Res Comm 11: 107- 112.

Olas, B., Wachowicz, B., Tomczak, A., Erler, J., Stochmal, A., Oleszek, W. (2008). Comparative anti platelet and antioxidant properties of polyphenol-rich extracts from: berries of *Aronia melanocarpa*, seeds of grape, bark of *Yucca schidigera in vitro*. Platelets, 19: 70-77.

Pankaj, P.P., Varma, M.C. (2013). Potential role of *Spirulina platensis* in maintaining blood parameters in alloxan induced diabetic mice. Int J Pharm Pharmaceut Sci 5: 450-456.

Pereira, C., Barros, L., Ferreira, I.C. (2016). Extraction, identification, fractionation and isolation of phenolic compounds in plants with hepatoprotective effects. J Sci Food Agric 96: 1068–1084.

Pereiraa, T.S., Sant'Annaa, J.R., Silvab, E.L., Pinheiroc, A.L., Alves, M.A. (2014). In vitro genotoxicity of Melaleuca alternifolia essential oil in human lymphocytes. J Ethnopharm 151: 852–857.

Petrovska, B.B. (2012). Historical review of medicinal plants' usage. Pharmacogn Rev 6: 1–5.

Price, E.A., Schrier, S.L. (2008). Anemia in the elderly: introduction. Semin Hematol 45: 207-209.

Reichling, J. (2010). Plant-Microbe Interactions and Secondary Metabolites with Antibacterial, Antifungal and Antiviral Properties, 2nd ed., Functions and Biotechnology of Plant Secondary Metabolites, Chapter 4, Annual Plant Reviews, Wink, M., Ed.,Wiley: Oxford, UK, 2010, Volume 39.

Saad, B., Azaizeh, H., Abu-Hijleh, G., Said, S. (2006). Safety of traditional Arab herbal medicine. Evidence Based complement. Altern Med 3: 433-439.

Saha, M.R., Hasana, S.M.R., Aktera, R., Hossaina, M.M., Alam, M.S., Alam, M.A., Mazumderc, M.E.H. (2008). In vitro free radical scavenging activity of ethanol extract of the leaves of *Mimusops elengi* linn. Bangl J Vet Med 6: 197–202.

Sayeed, M.A., Rashid, M.M., Kabir, M.F., Alam, R., Islamm S., Dharm R., Yusuf, A.T.M. (2014). In vitro anti-arthritic and thrombolytic activities of ethanolic extract of *Protium serratum* leaves. J Med Plant Res 8: 615-618.

Straus, J.H. (1998). Anaemia. In: Merck Veterinary Manual: A handbook of diagnosis, and therapy for Veterinarians. 8th ed. Merck and Co. Inc. Whitehouse Station, N. J. U.S.A. 8-18.

Sunmonu ,T.O., Oloyede, O.B. (2010). Performance and haematological indices in rats exposed to monocrotophos contamination. Human Exp Toxic 29:845-50.

Swenson, M.J., Reece, W.O. (1993). Duke's Physiology of Domestic Animals. 11th ed. Comstock Publishing Associates, Ithaca, New York, U.S.A.

Takshak, S., Agrawal, S.B. (2019). Defense potential of secondary metabolites in medicinal plants under UV-B stress. J Photochem Photobiol B Biol 193: 51–88.

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jager, A.K., van Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. Plant Growth Reg 34:23-37.

Tiwari, A.K. (2001). Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci 81: 1179-1187.

Tuerxun, H., Cui, J. (2019). The dual e_ect of morphine on tumor development. Clin Transl Oncol 21: 695–701.

Ughachukwu, P.O., Ezenyeaku, C.C.T., Onwurah, W.O., Ifediata, F. E., Ogamba J.O., Afonne, O.J. (2013). Effect on some haematological indices of human whole blood when aqueous leaf extract of *Euphorbia heterophylla* was used as storage anticoagulant. Af J Biotech 12: 4952-4955.

Ugwu,M.N., Umar, I.A., Utu-Baku, A.B., Dasofunjo, K., Ukpanukpong, R.U. (2013). Antioxidant status and organ function in streptozotocininduced diabetic rats treated with aqueous, methanolic and petroleum ether extracts of *Ocimum basilicum* leaf. Journal of Applied Pharmaceutical Science 3: 75-79

Wickens, G.E. (1998). Ecophysiology of economic plants in arid and semi-arid lands. Berlin: Springer.

Wickramasingh, S.N. (1991). Functions of red cells. In: Systemic Pathology 3rd edition, Churchill, Livingstone, Vol. 2 pp 6.

Wintrobe, M.M., Greer, J.P. (2009). Wintrobe's Clinical Hematology. Lippincott Williams & Wilkins, Philadelphia.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M.H., Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. Microb Pathog 124: 198–202.

Zlatic, N., Jakovljevic, D., Stankovic, M. (2019). Temporal, plant part, and inter population variability of secondary metabolites and antioxidant activity of *Inula helenium* L. Plants 8: 179.