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The efficacy of methanolic extract of herbal antioxidants from Cholistan desert for toxicological or phytomedicinal potential – An *in vitro* assay for human haematological attributes

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Secondary metabolites as non-enzymatic antioxidants are synthesized in desert plants which 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 antioxidants on hematological parameters can provide an insight for finding out their nature. Methanolic 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 Methanolic extracts of some plants from Cholistan desert of Pakistan were used to determine their effects on some human hematological attributes like counts for granulocyte; leukocytes; eosinophil; monocyte; lymphocyte; granuloytes and lymphocytes. Hemoglobin, Red Blood Cell, Mean Corpuscular Haemoglobin, Mean Corpuscular Hemoglobin Concentration, Mean Corpuscular Volume, Packed Cell Volume, Hematocrit and Red Cell Distribution were also studied. Methanolic extracts of plants collected from Cholistan 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 main characteristics of blood RBC and haemoglobin concentration was increased except by *Cressa* shoot and *Orobanche*stam and leaves extracts. Eosinophills, monocytes and platelets were decreases. *Citrulus* stem extract decreased RDW. Citrulus root extract decreased MPV. *Euphorbia* root extract decreased haemoglobin concentration. *Alhagii* shoot extract decreased lymphocytes percentage. MCHC was increased by extractions except by those of *Solanum, Euphorbia* and *Citrullus* root extracts caused reduction in MCHC.

Keywords: Efficacy, Methanol, Antioxidants, Desert plants, in vitro haematological indices

INTRODUCTION

Synthetic drugs resourced from medicinal plants are very important with minimum side effects (Andrade et al., 2019). Plants and their natural products not only are utilized for traditional medicines but nowadays they are being widely used in the production of commercial drugs. As reported on scientific and reliable evidences, 25% of the recommended medicines globally are derived plants (Benzie and Wachtel-Galor, 2011). According to report of WHO, about 80 percent of the world's population in Africa, Latin America,

Asia and the Middle East relies on plants primary healthcare needs (Rahman, 2014). Herbal medicines have minimal side effects and now a days millions of dollars are being invested by pharmaceutical companies to extract natural medicines from plants (Rahman, 2014; Alnafi, 2016). The increased demand for finding alternative medicines, has raised urge to evaluate plants for medicinal potentials.

There are substantial multitude pharmacological uses of plant extracts and plant extracts derived compounds (Oyenihi and Smith, 2019). Harnessing the biological potential of these medicinal plants represents them a novel therapeutic agent (Ayaz et al., 2019; Maher, 2019). Many medicinal plants are sources of antioxidants (Bitis et al., 2017). Therefore, research on antioxidants and their products is a significant topic in the medical field (Karihtala and Soini, 2007; Cheng et al., 2003). In human body many free radicals are formed like hydroxyl radical, superoxide radical and singlet oxygen etc collectively called as reactive oxygen species (ROS) (Weijl et al., 1997). Antioxidant enzymes, like catalase (CAT), superoxide dismutase (SOD) and peroxidases (GPx), remove free radicals and other ROS. Lowered antioxidant enzymes activities are considered to be associated with various pathologies. Plant extracts, essential oils and secondary metabolites are known as substances with antioxidant properties vital in the management of some diseases (Benzie and Wachtel-Galor, 2011; Rengasamy et al., 2019). Secondary metabolites has tremendous potential for use as drug (Dutt et al., 2019).

Neutrophil leukocytes of blood are a source of ROS (Dandona et al., 2000; Hatanaka et al., 2006). An array of increasing evidence is available for role of reactive oxygen species (ROS) in many diseases (Waddington et al., 2000; Sadzeviciene et al., 2006; Zekonis and Zekonis 2004; Chapple and Matthe, 2007).

There is an emerging interest for role of plant natural antioxidants in addition to their use as natural food additives and in cosmetics. Treating blood with plant extract containing antiuoxidants inhibits the release of reactive oxygen species by neutrophil leukocytes (Zekonis et al., 2008: Ielpo et al., 2000; Przyjemska and Zachwieja, 2005). Methanol extract contain high concentration of antioxidant as phenolic content.(Kabra et al., 2019;Manssouri et al., 2020) and exhibits more free radical scavenging activity than the other solvent based extracts plants. (Bagchi et al., 1997; Ashraf et al., 2020; Savadi et al., 2020). The extracts of plants show also significant effects on white blood cells such as lymphocytes, neutrophils, monocytes and eosinophils, and (Swenson and Reece, 1993). Plant extracts show increase in haemoglobin concentration and RBC count (Lohar et al., 2009;Sarswathi et al., 2007). Plants inhabiting the adverse habitat like deserts synthesize antioxidants for scavenging reactive oxygen species (ROS). Before preparation of any herbal product from these antioxidants we should have proper scientific studies on their safety and toxicity (Saad et al., 2006). Blood parameters are important in revealing the health status of an individual. Hence, the study of hematological attributes can be employed to evaluate the toxic or medicinal potentials of plant extracts (Sunmonu and Olyede, 2010).

MATERIALS AND METHODS

The choice of studies

This study is aimed to evaluated the toxic or medicinal nature of Methanol soluble phenolic antioxidants of Chloistan desert shrubs collected from Din Garh area. Toxic or medicinal nature of herbal extract is deduced by applying on animals (Ashafa, 2011). However, in vitro application of extracts on human hematological parameters directly, can be predictive for human utilization. To assessing medicinal or toxicological validation of phenolic extracts of desert plants Din Garh area of Cholistan was selected. Environment of desert enforces these plants to synthesize phenolic compounds for adaptation (Naz et al., 2010).The plants of desert have traditional utilization in the treatment of some diseases (Taylor et al., 2001). Moreover, the choice of in vitro human blood use is based on the physiological role blood plays in body and acts a valuable parameter of diagnosing a disease (Ganong, 2005). Hence, the investigation of various hematological parameters might be a useful index employed to assess the toxic or useful potentials of plant extracts containing antioxidants (Sunmonu and Oloyede, 2010).

Experimental design

Plant samples after collection, identification and necessary process were extracted in Methanol (Kinuthia et al 2014 and Saha, 2008). Blood was taken from a healthy volunteer was r mixing with plant extract (Ughachukwu et al., 2013 and Sayeed et al., 2014). Three samples of each extract were repeated to reduce the error.

Characteristics of blood were compared with normal blood and statistically then evaluated.

Field survey and trees sample collections

In a preliminary survey of Din Garh area of Cholistan desert geographical area and local plant names were known by meetings with local peoples. Specimens were collected and herborized. Herborized specimens were identified by specialists or by matching with labelled herbarium specimens lying in the departmental herbarium (Dr. Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakariya University, Multan Pakistan and/or the literature (Ali, 1993). Methodology of Jain, 1995 and Khan 1993 were employed for specimens collection keeping uniformity among age and size of plants and their parts. Further procedures were carried out in laboratory of the department.

Crude Methanolic extract preparation

The specimens were washed with running tap water followed by washing with 2% ethanol to remove dust and other surface contaminants. After drying them at room temperature, were grounded to fine powder using pestle and mortar. Following the procedure adopted by Afolayan et al.,(2010), crude Methanolic extract was prepared (100.0 g/200ml Methanol) by shaking at room temperature for 3h. After filtration, residue was repeated thrice for extraction.

Blood sampling and in vitro analysis

Human blood was obtained from a healthy volunteer of 25 years age having O^+ blood group after a questionnaire of not taking any medications or addictive substances and keeping a balanced diet (meat and vegetables); using no antioxidant supplements. By adding Methanol, plant extract was diluted up to 5ml. After consultation of 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).

RESULTS

***Citrullus colocynthis* stem**

Different sensitivity range was found in response of blood parameters treated with Methanolic extract. The promising role of extract, if the term may be used, in this fashion, was for leukocyte count (98.40%); monocyte (796.95%); granulocyte (3100%); granulocyte count (480%); lymphocyte count (54.10%); monocyte count (1415.39%); HGB (9.60%); HCT (7.34%); MCV (7.25%); MCH (9.30%) and MPV (8.23%). Although not statistically justified, but to a substantial level of increase has been observed in MCHC (6.95%) and RDW (1.09%). But the extract failed to maintain the trend of increment in some of the parameters. The significant decrease regarding role of extract was for lymphocyte (21.91%); eosinophils count (100%) and platelets (65.96%). Although not statistically justified, but to a substantial level of decrease has been shown by RBC (0.19%).

***Citrullus colocynthis* leaves**

The stimulating behavior of Methanolic extract varied considerably. The application of extract seems more promising in enhancing the granulocyte (24000%); granulocyte count (1100%); lymphocyte count (48.13%); HGB (10.80%); HCT (6.78%); MCV (8.47%) and MCH (11.16%).But the extract did not convincingly established a level of increment to a significant nature in RBC (0.58%); MCHC (5.52%) and RDW (2.26%). The application of extract seems to decreased significantly lymphocyte (42.44%); monocyte (100%); eosinophils (100%); eosinophils count (100%) and platelets (45.72%). But the extract did not convincingly establish a level of reduction to a significant nature in leukocyte count (3.02%) and monocyte count (100%).

***Citrullus colocynthis* root**

The marked increase was in granulocyte (24823.08%); lymphocyte (38.95%); granulocyte count (1146%); HGB (17.65%); HCT (19.65%); MCV (27.19%); MCH (16.74%) and RDW (9.84%). The increase was statistically negligible for lymphocyte count (31.34%) and RBC (1.94%). The observations are excluded from the ongoing trends for some of the parameters. The significant decrease was in monocyte (100%); eosinophils

(100%); eosinophils count (100%); platelets (50.70%) and MPV (6.64%). The increase was statistically negligible for monocyte (100%) and MCHC (4.78%) parameters

***Cressa critta* shoot**

Methanolic extract act as a potent factor in determining the change in blood parameters. Here it can be significantly discriminated that extract has played pivotal role in increasing granulocyte (33307.69%); monocyte (797.46%); granulocyte count (1520%); lymphocyte count (14.18%); monocyte count (669.23%); HGB (10.80%); HCT (7.48%); MCV (12.46%) and MCH (16.37%). Extract did not reveal statistically clear cut increase in leukocyte count (1.24%); MCHC (7.72%) and MPV (4.33%). Methanolic extract role deviated from these expectations of promotion in some of the parameters. Extract showed a decreased in lymphocyte (17.53%); eosinophils (100%); eosinophils count (99.76%); RBC (11.07%) and platelets (82.72%). Extract did not revealed statistically clear cut decrease in RDW (1.60%).

***Polygonum polgaloides* shoot**

Methanolic extract proved its marked influence when applied. Mean values showed enhancing role of Methanolic extract for leukocyte count (219.72%); monocyte (500.51%); granulocyte (29592.31%); granulocyte count (4280%); lymphocyte count (229.48%); monocyte count (1538.46%); HGB (14.41%); HCT (13.07%); MCV (10.65%) and MCH (19.84%). While some parameters did not show impressive response in this regard and increase was non-significant as RBC (1.94%) and MCHC (9.24%). An exception in this correlation was found in some of the parameters. Blood parameters, eosinophils (100%); eosinophils count (100%) and platelets (62.71%) revealed a significant decreased when treated with Methanolic extract. While some parameters did not showed impressive response in this regard and decrease was non-significant as lymphocyte (2.50%); RDW (1.38%) and MPV (1.59%).

***Orobanche aegyptiaca* leaves**

Methanolic extract substantially altered blood parameters. Extract showed appreciable rise in granulocyte (1100%); lymphocyte (38.33%); lymphocyte count (66.79%); eosinophils count (96.17%); HCT (8.23%); MCV (20.17%) and MCH (19.84%). The increase was statistically negligible for leukocyte count (19.00%); monocyte count (207.69%); HGB (6.48%); MCHC (3.72%); RDW (1.53%) and MPV (0.87%) parameters. But this was not consistent in all parameters. Extract showed a decreased in monocyte (148.73%); eosinophils (92.78%); RBC (11.45%) and platelets (49.92%).

***Orobanche aegyptiaca* stem**

Different sensitivity range was found in response of blood parameters treated with Methanolic extract. Exposure to extract has increasing impact of leukocyte count (37.30%); granulocyte (37538.46%); monocyte (620.81%); granulocyte count (2346%); monocyte count (746.15%); HCT (13.07%); MCV (18.35%); MCH (10.79%); and RDW (17.57%). Although not statistically justified, but a considerable extent of increase, was observed in lymphocyte count (4.10%) and MCHC (2.12%). But some parameters provided an opposite index. The significant decrease regarding role of extract was for lymphocyte (30.04%); eosinophils (88.09%); eosinophils count (93.30%); RBC (10.49%) and platelets (49.51%). Although not statistically justified, but a considerable extent of decrease was found in lymphocyte count (4.10%) and MCHC (2.12%).

***Euphorbia granulata* shoot**

Different sensitivity range was found in response of blood parameters treated with Methanolic extract. The application of extract seems promising in enhancing the granulocyte (1320%); lymphocyte (19.61%); granulocyte count (213%); lymphocyte count (55.60%); HCT (14.87%); MCV (21.07%) and MCH (11.16%). The increase was statistically negligible for leukocyte count (13.68%); monocyte count (230.77%); HGB (6.00%) and RDW (0.73%) parameters. Methanolic extract role deviated from these expectations of promotion in some of the parameters. Blood parameters, monocyte (224.87%); eosinophils (92.78%); eosinophils count (96.42%) and platelets (65.96%) revealed a significant decreased when treated with Methanolic extract. Although not statistically justified, but to a substantial level of decrease was observed in RBC (4.08%); MCHC (30.20%) and MPV (4.76%).

Table: 1 In vitro assay the efficacy of Methanolic extract of antioxidants of herbs from Cholistan desert for for human haematological attributes (values expressed mean±standard error parenthesis values represent percentage difference over control group (-) sign shows high value than normal value; n=3)

Species	Leukocyt count 103/µL (1.24)	Granulocyte %age(3.41)	Lyrnphocyte %age(3.24)	Monocyte %age (1.26)	Eosinphils %age (0.07)	Granulocyte count 10^3/µL (0.84)	Lymphocyte count 10^3/µL (0.82)	Monocyte count 10^3/µL (0.33)	Eosinophils count 10^3/µL (0.40)
Normal blood	5.63±0.06 ijkl	0.13±0.06 q	47.93±0.06 H	1.97±0.06 j	2.77±0.06 a	0.15±0 j	0.68±0.03 ijklm	0.13±0.06 gh	50.33±0.58 a
***Citrullus colocynthis* stem**	11.17±0.76 c (-98.40)	44.7±1.2 f (-34284.62)	37.43±1.40 jkl (21.91)	17.67±0.61 d (-796.95)	0±0e(100)	4.8±0.75 cd (-3100)	4.13±0.65 defg (-54.10)	1.97±0.45 c (-1415.39)	0±0f(100)
***Citrullus Colocynthis* leaves**	5.8±0.1 ghijkl (3.02)	31.33±1.39 jk (-24000)	68.27±3.25 c (42.44)	0±0k(100)	0±0e(100)	1.8±0.3 h (-1100)	3.97±0.45 defg (-48.13)	0±0h(100)	0±0f(100)
***Citrullus colocynthis* root**	5.43±0.06 jkl (3.55)	32.4±3.15 ij (-24823.08)	66.6±1.25 cd (-38.95)	0±0k(100)	0±0e(100)	1.87±0.40 h (-1146)	3.52±0.48 efghij (-31.34)	0±0h(100)	0±0f(100)
***Cressa cretica* shoot**	5.7±0.6 hijkl (-1.24)	43.43±1.11 f (-33307.69)	39.53±1.72 jk (17.53)	17.68±0.75 d (-797.46)	0±0e(100)	2.43±0.25 gh (-1520)	2.3±0.1lm (-14.18)	1±0.1e(-669.23)	0.12±0.08 f (99.76)
***Polygonum pollogonoides* shoot**	18±0.56 b (-219.72)	38.6±4.35 g (-29592.31)	46.73±0.67 hi (2.50)	11.83±0.80 f (-500.51)	0±0e(100)	6.570±1.01 b (-4280)	8.83±0.25b (-229.48)	2.13±0.35bc (-1538.46)	0±0f(100)
***Orobanche aegyptiaca* leaves**	6.7±0.35 efghij (-19.00)	26.13±3.66 lm (-20000)	66.3±4.56 cd (-38.33)	4.9±0.66 i (-148.73)	0.2±0.1 cd (92.78)	1.8±0.2 h (-1100)	4.47±0.45 de (-66.79)	0.4±0.1fgh (-207.69)	1.93±0.40 d (96.17)
***Orobanche aegyptiaca* stem**	7.73±0.46 def (-37.30)	48.93±4.35 e (-37538.46)	33.53±3.85 mno (30.04)	14.2±1.25 e (-620.81)	0.33±0.15 b (88.09)	3.67±0.35 ef (-2346)	2.57±0.06 jklm (4.10)	1.1±0.2de (-746.15)	3.37±0.40 c (93.30)

Values sharing the same letters in respective column differ non-significantly; The values in parenthesis in the 1st row represents LSD value; Leukocyte count= WBC; Continuoue….

Table: 1 (continue…) In vitro assay the efficacy of Methanolic extract of antioxidants of herbs from Cholistan desert for for human haematologicalattributes (values expressed mean±standard error parenthesis values represent percentage difference over control group (-) sign shows high value than normal value; n=3)

Species	RBC 10^6/µL (0.35)	HGB g/dL (0.69)	HCT %age (1.20)	MCV FL (2.53)	MCH PG (1.15)	MCHC %age (4.73)	RDW %age (0.73)	Platelets 10^3/L (68.40)	MPV 10^3/µL (0.39)	RDW 10^3/µL (0.56)
Normal blood	5.15±0.01 a	8.33±0.06 fghi	22.73±0.06 k	44.13±0.06 l	16.13±0.06 lm	34.97±0.85 cde	13.72±0.03 ghi	642.33±0.58 a	6.93±0.3 Efg	17.33±0.06 fghi
***Citrullus colocynthis*stem**	5.14±0.002 a (0.19)	9.13±0.35 cdef (-9.60)	24.4±0.6 fghi (-7.34)	47.33±1.26 ijk (-7.25)	17.63±0.60 hij (-9.30)	37.4±0.46 abcde (-6.95)	13.87±0.06 ghi (-1.09)	218.67±25.11 i (65.96)	7.5±0.3 abc (-8.23)	17.07±0.15 jk (1.95)
***Citrullus colocynthis* leaves**	5.18±0.02 a (-0.58)	9.23±0.25 bcde (-10.80)	24.27±0.60 fghij (-6.78)	47.87±1.27 ijk (-8.47)	17.93±0.15 hij (-11.16)	36.9±0.1 bcde (-5.52)	14.03±0.25 efghi (-2.26)	348.67±49.00 d (45.72)	7.27±0.15 abcdef (-4.91)	17.97±0.25 cdefghi (-3.22)
***Citrullus\ colocynthis*root**	5.25±0.05 a (-1.94)	9.8±0.75 bcd (-17.65)	29.47±0.90 a (-19.65)	56.13±1.98 a (-27.19)	18.83±0.80 efgh (-16.74)	33.3±0.75 e (4.78)	15.07±0.70 c (-9.84)	316.67±62.52 defg (50.70)	6.47±0.25 hi (6.64)	18.23±0.45 cdef (-4.71)
***Cressa cretica*shoot**	4.58±0.62 def (11.07)	9.23±0.25 bcde (-10.80)	24.43±0.55 fghi (-7.48)	49.63±0.70 defghi (-12.46)	18.77±0.40 efgh (-16.37)	37.67±0.55 abcde (-7.72)	13.5±0.1 hi (1.60)	111±25.94 i (82.72)	7.23±0.15 bcdef (-4.33)	18.53±0.45 cd (-6.43)
***Polygonum pollogonoides* shoot**	5.25±0.05 a (-1.94)	9.53±0.25 bcde (-14.41)	25.7±0.8 def (-13.07)	48.83±0.76 fghij (-10.65)	19.33±0.65 defg (-19.84)	38.2±0.75 abcde (-9.24)	13.53±0.06 hi (1.38)	243±55.03 ghi (62.17)	6.82±0.03 fgh (1.59)	17.9±0.1 cdefghi (-2.81)
***Orobanche aegyptiaca* leaves**	4.56±0.51 ef (11.45)	8.87±0.15 efgh (-6.48)	24.6±0.95 fgh (-8.23)	53.03±4.42 bc (-20.17)	19.33±1.46 defg (-19.84)	36.27±0.15 cde (-3.72)	13.93±0.15 fghi (-1.53)	321.67±36.07 defg (49.92)	6.87±0.06 fgh (-0.87)	17.87±0.15 defghi (-2.64)
***Orobanche aegyptiaca* stem**	4.61±0.56 cdef (10.49)	8.83±0.25 efgh (-6.00)	25.7±1.05 def (-13.07)	52.23±3.45 bcd (-18.35)	17.87±0.35 hij (-10.79)	34.23±1.20 de (2.12)	16.13±0.85 b (-17.57)	324.33±24.50 defg (49.51)	6.87±0.6 fgh (-0.87)	17.87±0.15 defghi (-2.64)

Values sharing the same letters in respective column differ non significantly; The values in parenthesis in the 1st row represents LSD value; RBC= Red Blood Cells: HGB= Haemoglobin; HCT= Hematocrit; MCV= Mean Carpuscular Volume; MCH= Mean CarpuscularHaemoglobin; MCHC= Mean CarpuscularHaemoglobin concentration, RDW= Red Cell Distribution Width; Platelets= thrombocyte; MPV= Mean Platelet Volume

Table: 2 In vitro assay the efficacy of Methanolic extract of antioxidants of herbs from Cholistan desert for for human haematological attributes (values expressed mean±standard error parenthesis values represent percentage difference over control group (-) sign shows high value than normal value; n=3)

Species	Leukocyte count 103/µL (1.24)	Granulocyte %age (3.41)	Lyrnphocyte %age (3.24)	Monocyte %age (1.26)	Eosinphils %age (0.07)	Granulocyte count 10^3/µL (0.84)	Lymphocyte count 10^3/µL (0.82)	Monocyte count 10^3/µL (0.33)	Eosinophils count 10^3/µL (0.40)
Normal blood	5.63±0.06 ijkl	0.13±0.06q	47.93±0.06h	1.97±0.06j	2.77±0.06a	0.15±0j	0.68±0.03 Ijklm	0.13±0.06 gh	50.33±0.58a
***Euphorbia granulata* shoot**	6.4±0.03 fghijk (-13.68)	34.13±3.01 hij (-26153.85)	57.33±1.70 fg (-19.61)	6.4±0.4h (-224.87)	0.2±0.1cd (92.78)	2.13±0.40h (-1320)	4.17±0.60 defg (-55.60)	0.43±0.15fg (-230.77)	1.8±0.5d (96.42)
***Euphorbia granulata* root**	5.62±0.6 ijkl (0.18)	35.47±1.43 ghi (-27184.62)	60.03±3.27 ef (-25.25)	0±0k(100)	0±0 e (100)	2±0.3 h (-1233)	3.57±0.45 efghi (-33.21)	0±0h(100)	0±0f(100)
***Alhagii maurorum* shoot**	6.9±0.25 efghij (-22.56)	67.17±1.86 a (-51569.23)	33.2±2.21 mno (30.73)	0±0k(100)	0±0 e (100)	3.93±0.70 def (-2520)	2.3±0.1 lm (14.18)	0±0h(100)	0±0f(100)
***Solanum xanthocarpus* leaves**	4.77±0.03l (15.28)	15.1±1.05o (-11515.38)	84.43±1.29 a (-76.15)	0±0k(100)	0±0 e (100)	0.67±0.15 ij (-1690)	4.17±0.60 defg (-55.60)	0±0h(100)	0±0f(100)
***Solanumxan thocarpus*root**	5.52±0.2 jkl (1.95)	44.2±2.03f (-33900)	55.03±1.68g (-14.81)	0±0k(100)	0±0 e (100)	2.5±0.5 gh (-1566)	2.97±0.06 hijklm (-10.82)	0±0h(100)	0±0f(100)
***Solanum surattense* leaves**	5.2±0.06 kl (7.64)	36.73±0.80 gh (-28153.85)	63.4±1.61de (-32.28)	0±0k(100)	0±0 e (100)	1.87±0.35 h (-1146)	3.27±0.25 ghijkl (-22.01)	0±0h(100)	0±0f(100)

Values sharing the same letters in respective column differ non-significantly; The values in parenthesis in the 1st row represents LSD value; Leukocyte count= WBC; Continuoue….

Table: 2 (continue) In vitro assay the efficacy of Methanolic extract of antioxidants of herbs from Cholistan desert for for human haematological attributes (values expressed mean±standard error parenthesis values represent percentage difference over control group (-) sign shows high value than normal value; n=3)

Species	RBC 10^6/µL (0.35)	HGB g/dL (0.69)	HCT %age (1.20)	MCV FL (2.53)	MCH PG (1.15)	MCHC %age (4.73)	RDW %age (0.73)	Platelets 10^3/L (68.40)	MPV 10^3/µL (0.39)	RDW 10^3/µL (0.56)
Normal blood	5.15±0.01 a	8.33±0.06 Fghi	22.73±0.06 k	44.13±0.06 l	16.13±0.06 lm	34.97±0.85 cde	13.72±0.03 ghi	642.33±0.58 a	6.93±0.3 Efg	17.33±0.06 fghi
***Euphorbia granulata* shoot**	4.94±0.07 abcdef (4.08)	8.83±0.25 efgh (-6.00)	26.11±1.17 cde (-14.87)	53.43±0.55 b (-21.07)	17.93±0.60 hij (-11.16)	45.53±0.60 de (30.20)	13.82±0.03 ghi (-0.73)	218.67±23.46 i (65.96)	6.6±0.1 ghi (4.76)	18.27±0.25 cdef (-4.94)
***Euphorbia granulata* root**	4.63±0.09 bcdef (10.10)	8.13±0.06 hi (2.40)	22.87±0.06 jk (-0.62)	49.2±0.75 efghij (-11.49)	17.43±0.55 hijk (-8.06)	35.47±0.50 cde (1.43)	13.78±0.03 ghi (-0.44)	333.33±39.02 def (48.11)	7.2±0.1 bcdef (-3.90)	17.48±0.03 ghij (-0.40)
***Alhagii maurorum* shoot**	4.87±0.14 abcdef (5.44)	9.13±0.35 cdef (-9.60)	25.23±0.70 efg (-11.00)	51.63±1.46 bcdef (-17.00)	19.47±0.75 def (-20.71)	37.5±0.6 abcde (-7.23)	14.37±0.15 cdefgh (-4.74)	289±32.60 defghi (55.01)	7.1±0.1 bcdef (-2.45)	17.42±0.03 ghij (-0.06)
***Solanum xanthocarpus* leaves**	5.07±0.03 ab (1.56)	10.07±0.60 ab (-20.89)	26.47±0.50 cde (-16.45)	51.53±1.82 bcdefg (-16.77)	19.73±0.65 de (-22.32)	37.8±0.75 abcde (-8.10)	13.87±0.06 ghi (-1.09)	250.33±47.65 ghi (61.03)	7.27±0.25 abcdef (-4.91)	18.07±0.40 cdefg (-3.79)
***Solanum xanthocarpus*root**	4.85±0.05 abcdef (5.83)	8.13±0.06 hi (2.40)	23.53±0.60 hijk (-3.52)	48.6±1.51 ghij (-10.13)	16.7±0.2 jklm (-3.53)	34.43±0.51 de (1.54)	14.87±0.65 cde (-8.38)	340±51.57 de (47.07)	6.98±0.03 defg (-0.72)	18.43±0.45 cdef (-5.86)
***Solanum surattense* leaves**	4.86±0.06 abcdef (5.63)	8.13±0.06 hi (-2.40)	22.87±0.55 jk (-0.62)	47.37±0.81 ijk (-7.34)	16.03±0.25 lm (0.62)	36.2±0.46 cde (3.52)	14.53±0.50 cdefg (-5.90)	293±23.07 defghi (54.38)	6.98±0.03 defg (-0.72)	17.42±0.03 ghij (-0.06)

Values sharing the same letters in respective column differ non significantly; The values in parenthesis in the 1st row represents LSD value; RBC= Red Blood Cells: HGB= Haemoglobin; HCT= Hematocrit; MCV= Mean Carpuscular Volume; MCH= Mean CarpuscularHaemoglobin; MCHC= Mean CarpuscularHaemoglobin concentration, RDW= Red Cell Distribution Width; Platelets= thrombocyte; MPV= Mean Platelet Volume

Euphorbia granulata root

The stimulating behavior of Methanolic extract varied considerably. The marked increase was in granulocyte (1233%); lymphocyte (25.25%); granulocyte count (200%); MCV (11.49%) and MCH (8.06%).

Extract did not revealed statistically clear cut increase in leukocyte count (0.18%); lymphocyte count (33.21%); HCT (0.62%); RDW (0.44%) and MPV (3.90%). An exception in this correlation was found in some of the parameters. The significant decrease regarding role of extract was for monocyte (100%); eosinophils (100%); eosinophils count (100%); RBC (10.10%) and platelets (48.11%). Extract did not revealed statistically clear cut decrease in monocyte count (100%) and MCHC (1.43%)

***Alhagii maurorum* shoot**

Here it can be significantly discriminated that extract has played pivotal role in increasing leukocyte count (22.56%); granulocyte (2520%); granulocyte count (393%); HGB (9.60%); HCT (11.00%); MCV (17.00%) and MCH (20.71%). While some parameters did not showed impressive response in this regard and increase was non-significant as leukocyte count (0.18%); lymphocyte count (33.21%); MCHC (7.23%); RDW (4.74%) and MPV (2.45%). But this was not consistent in all parameters. The application of extract seems to decreased significantly lymphocyte (30.73%); monocyte (100%); eosinophils (100%); eosinophils count (100%) and platelets (55.01%). While some parameters did not showed impressive response in this regard and decrease was non-significant as lymphocyte count (14.18%); monocyte count (100%) and RBC (5.44%)

***Solanum xanthocarpus* leaves**

Exposure to extract has increasing impact on granulocyte (1690%); lymphocyte (76.15%); lymphocyte count (55.60%); HGB (20.89%); HCT (16.45%); MCV (16.77%); MCH (22.32%) and platelets (61.03%). Extract did not revealed statistically clear cut increase in leukocyte count (15.28%); MCHC (8.10%) and RDW (1.09%). Methanolic extract affected some parameters with different degrees thereby lowering values from control. Extract showed a decreased in monocyte (100%); eosinophils (100%); eosinophils count (100%) and platelets (61.03%). Although not statistically justified, but to a substantial level of decrease was found in monocyte count (100%) and RBC (1.56%).

***Solanum xanthocarpus* root**

Methanolic extract act as a potent factor in determining the change in blood parameters. Extract increased granulocyte (1566%); lymphocyte (14.81%); MCV (10.13%) and RDW (8.38%). While some parameters did not showed impressive response in this regard and increase was non-significant as leukocyte count (1.95%); lymphocyte count (10.82%); HCT (3.52%); MCH (3.53%) and MPV (0.72%). Methanolic extract role deviated from these expectations of promotion in some of the parameters. The significant decrease was in monocyte (100%); eosinophils (100%); eosinophils count (100%) and platelets (47.07%). Although not statistically justified, but to a substantial level of decrease has been observed in monocyte count (100%); RBC (5.83%); MCH (0.62%) and MCHC (3.52%).

***Solanum surattense* leaves**

Methanolic extract substantially altered blood parameters. Index of variability in blood parameters revealed a significant difference in granulocyte (1146%); lymphocyte (32.28%); granulocyte count (187%) and MCV (7.34%). While some parameters did not showed impressive response in this regard and increase was non-significant for leukocyte count (7.64%); lymphocyte count (22.01%); HGB (2.40%); HCT (0.62%); RDW (5.90%) and MPV (0.72%).Metanolic extract affected some of the parameters with different degrees thereby lowering values from control. Extract showed a decreased in monocyte (100%); eosinophils (100%); eosinophils count (100%) and platelets (54.38%). No remarkable variation in monocyte count (100%); RBC (5.63%); MCH (0.62%) and MCHC (3.52%).

DISCUSSION

Phenolic compounds, play important role against reactive oxygen species (Pang et al., 2018). Having protective effects when present in plants used as food (Niciforovic et al.,2010). Phenolics such as Anastatins A and B and others isolated from desert plant are reported to have protection for hepatocytes (Xiang et al., 2020). The antioxidant potential of phenols extracted from plants is effective in control of cardiovascular disease and cancer (Duthieet al., 2000; Li et al., 2014; Balmus et al., 2016). It is reported that phenolic content are extracted more in solvent with higher polarity (Barchan et al.,

2014; Belyagoubi et al., 2016). Methanol extract contain high concentration of antioxidant as phenols (Kabra et al., 2019;Manssouri et al., 2020) and has more ROS scavenging ability than the other solvent extracts (Bagchi et al., 1997; Ashraf et al., 2020; Savadi et al., 2020). This ability of ROS scavenging change the blood attributes when added in the form of plant extracts.

Xerophytes of the Cholistan desert are adapted to various stresses by production of antioxidants which used by the local inhabitants of Cholistan as folk remedies. Our results revealed an array of diverse effects of Methanolic extracts of different plant parts on hematological attributes. The results are in harmony with the earlier findings of Swenson and Reece, 1993; Olson et al., 1984 and Straus, 1998 while in contradict to the findings of Lohar et al., (2009) and Owoyele, (2003) regarding RBC and haemoglobin concentration (MCH, MCHC). This diversity might be according to the opinion of some of the researchers that some herbal plants are often non-specific in their actions (Treasure, 2000).

Phenolic compounds as antioxidants are the most abundant in desert plants (Fiorentino et al., 2006). Antioxidants act as cell saviors by free radical scavenging activity (Fattouch et al., 2007). Being antioxidants, flavonoids are especially important for protection against human diseases (Tiwari, 2001).

Results revealed that Methanolic extracts of leaf, stem and flower reduced the RBC (Table.1&2). Red Blood Cells (Erythrocytes) are the most abundant cells in the human body possessing physiological importance. Erythrocytes are more susceptible to medicines (Hamidi and Tajerzadeh, 2003). Erythrocytes are also major target of free radicals (ROS) owing to the presence of both high concentrations of membrane polyunsaturated fatty acids (Ebrahimzadeh et al., 2009). Oxidative damage to the erythrocyte membrane may be due to hemolytic activity of ROS. The hemolysis of red blood cells by ROS destructs 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 episode of the lysis of cell (Devjani and Verma, 2010).

Results revealed that Methanolic extracts of leaf, stem and flower decreased MCHC and increased MCH and MCV (Table.1&2). 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 each red blood cell (Green, 1978). An increased level of MCV accompanied with a reduction in MCHC might bedue to osmotic fragility of the cell membrane (Olaleye, 2007). Haemoglobin and RBC are associated with the total numbers of red blood cells while MCV, MCH and MCHC relates to individual red blood cells (Ashafa, 2011). The MCV is for the size of the RBCs. When the MCV is high, the RBCs will be larger than normal and are termed as macrocytic. When the MCV is below normal, the RBCs will be smaller than normal and are called as microcytic. RBCs of normal size are termed normocytic. A reduction in MCV acoounts for its use or interference with iron uptake by hemoglobin. Furthermore, decrease in the blood cells may be due to the increased glycosylation (non-enzymatic) of membrane proteins which can also cause hyperglycemia. Oxidation of glycosylated membrane proteins and hyperglycemia causes hemolysis of cells (Crouch et al., 1981).

Some samples reduced the haemoglobin concentration while others induced an enhancement (Table.1&2). Change in haemoglobin concentration might be due to iron deficiency in it. Iron deficiency could be due to interference of molecules with iron or its metabolism.

There was reduction in platelets by Methanolic extracts of some specimens (Table.1&2). Blood platelet might be changed by antiplatelet activity of antioxidants (Dutta-Roy, 2002). Change in Blood platelet might be by platelets adhesion to collagen and platelet aggregation by ROS species in resting blood platelets. Platelets reduction may be beneficial at some level because platelets reduce the blood viscosity which correlates positively to blood pressure and may be beneficial in terms of the clinical haematology. Increase in haemoglobin concentration (MCH, MCHC) among all the haemotological parameters may be due to the presence of active principles that stimulate haemopoiesis, or support in availability of iron for haemopoiesis, or agents for chelating iron are may be weakly present or completely absent in the plant extract which decreased the hemolysis of RBC (Lohar et al., 2009). The increase in haemoglobin (MCH and MCHC) causes oxygen transport to the tissues (Gruchy, 1976). An increase or decrease in blood attributes might be owed to free radical scavenging activity of extract (Saha et. al. 2008);anticoagulation by extract

(Ughachukwu et.al. 2013); antiuglycosylation (Nair et.al. 2013); thrombolytic potential (Sayeed et.al. 2014) or by genotoxicity (Pereira et al., 2014).

CONCLUSION

Antioxidant extracts have their effects on haematology and should be used with care and after extensive exploration of their toxic nature.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MS and GY designed and conducted experiment. AM and SM contributed in writing the manuscript. IA conducted proof reading.

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REFERENCES

Afolayan AJ, Aboyade OM, Adedapo AA, Sofidiya OM, 2010. Anti-inflamatory and analgesic activity of the Methanol extract of *Malva parviflora* Linn. (Malvaceaea) in rats.Af J Biot 9: 1225-1229.

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

Alnafi PDAE, 2016. A review on chemical constituents and pharmacological activities of *Coriandrum sativum*.IOSR J Pharm 6: 17–42.

Andrade S, Ramalho MJ, Loureiro JA Pereira MD, 2019. Natural compounds for lzheimer's disease therapy: a systematic review of preclinical and clinical studies. Int J Mol Sci 20: 41.

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

Ashraf K, Halim H, Lim SM, Ramasamy K, Sultan S, 2020. *In vitro* antioxidant, antimicrobial and antiproliferative studies of four different extracts of *Orthosiphon stamineus*, *Gynura procumbens* and *Ficus deltoidea.*Saudi J BiolSci 27: 417-432.

Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, Devkota HP, 2019. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. Chemico-BiolInterac 308: 294–303.

Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ, 1997. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. Res Commun Mol Pathol Pharmacol 95:179-189.

Balmus I, Ciobica A, Trifan A, Stanciu C, 2016. The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. Saudi J Gastroenterol 22: 3–17.

Barchan A, Bakkali M, Arakrak A, Pagan R Laglaou A, 2014. The effects of solvents polarity on the phenolic contents and antioxidant activity of three Mentha species extracts.Int J Curr Microbiol Appl Sci 3: 399–412.

Belyagoubi L, Belyagoubi-Benhammou N, Coustard JM, 2016.Effects of extraction solvents on phenolic content and antioxidant properties of *Pistacia atlantica*.Desf fruits from Algeria.Int Food Res J 23: 948–953.

Benzie IFF, Wachtel-Galor S, 2011.Herbal Medicine: Biomolecular and Clinical Aspects, CRC Press/Taylor & Francis Group, Boca Raton, FL, USA, 2nd edition, 2011.

Bitis L, Sen A, Ozsoy N, 2017. Flavonoids and iological activities of various extracts from Rosa sempervirens leaves. Biotech Equip 31:299–303.

Chapple IL, Matthews JB, 2007. The role of reactive oxygen and antioxidant species in periodontal tissue destruction.Periodontol 43:160-232.

Cheng HY, Lin TC, Yu KH, 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula.*Biol Pharmac Bull 26:1331–1335.

Crouch RK, Gandy SE, Kimsey GA, Richard, Galbrath, A.G. Galbrath 1981. desert, Pakistan. Pak J Bot 42:839-851.

Dandona P, Karne R, Ghanim H, Hamouda W, Aljada A, Magsino CH, 2000. Cardilol inhibits reactive oxygen species generation by leukocytes and oxidative damage to amino acids. Circulation 101:122-124.

Devjani C, Verma RJ, 2010. Ameliorative effect of *Emblica officinalis* aqueous extract against ochratoxin – induced lipid peroxidation in the kidney and liver of mice.Int J Occup Med Environ Health 23: 1 – 11.

Duncan DB, 1955. Multiple Range and Multiple F-Test. Biometrics 11: 1-42.

Duthie GG, Duthie SJ, Kyle JAM, 2000. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr Res Rev 13: 79.

Dutt R, Garg V, Hatri N, Madan AK, 2019.Phytochemicals in anticancer drug development.Anti-Cancer Agents in Med Chem 19:172–183.

Dutta-Roy AK, 2002. Dietary components and human platelet activity.Platelets 13:67-75.

Ebrahimzadeh MA, Ehsanifar S, Eslami B, 2009. *Sambucus ebuluselburensis* fruits: A good source for antioxidants. Phcog Mag 4: 213-218.

Fattouch S, Caboni P, Coroneo V, Tuberoso CIG, 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.

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

Green JH, 1978. An Introduction to Human Physiology. African edition, Oxford University Press, Ibadan.

Gruchy GC, 1976. The red cell anaemia. In, Clinical Haematology in Medical Practice. 3rd edition, Blackwell Scientific Publications, Oxford univ.

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

Hatanaka E, Levada-Pires AC, Pithon-Curi TC, Curi R, 2006. Systematic study on ROS production induced by oleic, linoleic, and gamma-linolenic acids in human and rat neutrophils. Free Radic Biol Med 41:1124-32.

Ielpo MT, Basile A, Miranda R, Moscatiello V, Nappo C, Sorbo S, 2000. Immunopharmacological properties of flavonoids. Fitoterapia 71:101-109.

Jain SK, 1995. A Manual of Ethnobotany, 2nd Edition. Scientific Publishers, Jodhpur.

Kabra A, Sharma R, Hano C, Kabra R, Martins N, Baghel US, 2019. Phytochemical Composition, Antioxidant, and Antimicrobial Attributes of Different Solvent Extracts from *Myrica esculenta* Buch.-Ham. ex. D. Don Leaves. Biomol 9: 357.

Karihtala P, Soini Y, 2007. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. PMIS 115:81–103.

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

Kinuthia GK, Kabiru EW, Anjili CO, Kigondu EM, Ngure VN, Ingonga JM, Gikonyo IK, 2014. Efficacy of crude methanolic extracts of *Allium sativum* L. and *Moringa stenopetala* (Baker f.) Cufod. Against *Leishmania major*. Intr J Med Aroma Plants 4: 16-25.

Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB, 2014. Resources and biological activities of natural polyphenols. Nutrients 6: 6020–6047.

Lohar PS, lohar MS, Roychoudhury S, 2009. Erythropoitic effects of some medicinal plants of india on experimental rat model. Slovak J Anim Sci 42: 95–98.

Maher P, 2019. The potential of flavonoids for the treatment of neurodegenerative diseases. Int J Mol Sci 20: 3056.

Manssouri M, Znini M, Majidi L, 2020. Studies on the antioxidant activity of essential oil and various extracts of *Ammodaucus leucotrichus* Coss. & Dur. Fruits from Morocco. J Taibah Univ Sci 14:124-130.

Nair SS, 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 MSA, Ashraf M, Arshad M, 2010. Is soil salinity one of the major determinants of community structure under arid environments? Commun Ecol 11: 84-90.

Niciforovic N, Mihailovic V, Maskovic P, Solujic S, Stojkovic A, Muratspahic DP, 2010. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem Toxicol 48: 3125–3130.

Olaleye SB., Iranloye BO, Salami HA, Elegbe RA, 1999. Erythrocyte osmotic fragility and other

blood parameters in rats fed with diets containing raw and processed soyabeans (*Glycine max*). Biosci Biotech 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.

Olson CT, Keller WC, Gerken DF, Reed SM, 1984. Suspected tremetol poisoning in horses. J Anim Vet Med Ass 185: 1001-1003.

Oyenihi AB, Smith C, 2019. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success. J Ethnopharm 229: 54–72.

Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y, Bao J, 2018. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chem 240: 212–221.

Pereiraa TS, Sant'Annaa JR, Silvab EL, Pinheiroc A, Alves MA, 2014. In vitro genotoxicity of *Melaleuca alternifolia* essential oil in human lymphocytes. J Ethnopharm 151: 852–857.

Przyjemska MZ, Zachwieja AD, 2005. Oxidative metabolism of neutrophils in obese patients before and during body mass reduction: the *in vitro* effect of quercetin and rutin. Pol Arch Med Wewn 113:231-40.

Rahman S, 2014. *Cynodon Dactylon*: Antimicrobial Potential of Crude Extract as Valuable Medicinal Plant, BRAC University, Bangladesh, India, 2014.

Rengasamy KRR, Khan H, Gowrishankar S, 2019. The role of flavonoids in autoimmune diseases: therapeutic updates. Pharmaco Therap 194:107–131.

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

Sadzeviciene R, Zekonis J, Zekonis G, Paipaliene P, 2006. Oxidative function of neutrophils in periodontitis patients with type 1 diabetes mellitus. Medicina (Kaunas) 42:479-483.

Saha MR, Hasana SMR, Aktera R, Hossaina MM, Alam MS, Alam MA, Mazumderc MEH, 2008. I*n vitro* free radical scavenging activity of methanol extract of the leaves of M*imusops elengi* linn. Bangl J Vet Med 6: 197–202.

Sarswathi R, Neelaveni T, Swetha N, Priyadarshani R, 2007. Formulation and evaluation of polyhedral capsule for iron deficiency anaemia. Hamdard Medicus 50: 92-99.

Savadi S, Vazifedoost M, Didar Z, Nematshahi MM, Jahed E, 2020. Phytochemical Analysis and Antimicrobial/Antioxidant Activity of *Cynodon dactylon* (L.) Pers. Rhizome Methanolic Extract. J Food Qual Article ID 5946541, 10 pages.https://doi.org/10.1155/2020/5946541

Sayeed MA, Rashid MM, Kabir MF, Alam R, Islam S, Dhar R, Yusuf ATM, 2014. In vitro anti-arthritic and thrombolytic activities of methanolic extract of *Protium serratum* leaves. J Med Plant Res 8: 615-618.

Straus JH, 1998. Anaemia. In, Merck Veterinary Manual, A handbook of diagnosis, and therapy for Veterinarians. 8th ed. Merck and Co. Inc. Whitehouse Station, N.J. USA.pp: 8-18.

Sunmonu TO, Oloyede OB, 2010. Performance and haematological indices in rats exposed to monocrotophos contamination. Human Exp Toxic 29:845-50.

Swenson MJ, Reece WO, 1993. Duke's Physiology of Domestic Animals. 11th ed. Comstock Publishing Associates, Ithaca, New York, USA.

Taylor JLS, Rabe T, McGaw LJ, Jager AK, van Staden J, 2001. Towards the scientific validation of traditional medicinal plants. Plant Growth Reg 34:23-37.

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

Treasure J, 2000. Medical Herb (online) http://www.herbological.com.

Ughachukwu PO, Ezenyeaku CCT, Onwurah WO, Ifediata F E, Ogamba JO, Afonne OJ, 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.

Waddington RJ, Moseley R, Embery G, 2000. Review. Periodontal disease mechanisms. Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. Oral Dis 6:138-151.

Weijl NI, Cleton FJ, Osanto S, 1997. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat Rev 23:209–240.

Wickramasingh SN, 1991. Functions of red cells.

In: Systemic Pathology 3rd edition, Churchill, Livingstone. Pp: 6.

Xiang C, Cao M, Miao A, Gao F, Li X, Pan G, Zhang W, Zhang Y, Yu P, Teng Y, 2020. Antioxidant activities of anastatin A & B derivatives and compound 38c's protective effect in a mouse model of CCl_4-induced acute liver injury. RSC Adv pp: 14337-14346.

Zekonis G, Zekonis J, 2004. Effect of bacterial stimulants on release of reactive oxygen metabolites from peripheral blood neutrophils in periodontitis. Medicina (Kaunas) 40:260-264.

Zekonis G, Zekonis J, Sadzeviciene R, Simoniene G, Kevelaitis E, 2008. Effect of Perilla frutescens aqueous extract on free radical production by human neutrophil leukocytes. Medicina (Kaunas) 44: 699-704.