

PHARMACODYNAMICS OF PHENOLICS RICH EXTRACT OF SHRUBS FROM CHOLISTAN DESERT: HAEMATOLOGICAL INDICES

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ABSTRACT

Antioxidants resourced from stress adapted plants have medicinal properties or be toxic. Practical utilization of these as medicines need to explore the exact nature of discrimination of their actions. By testing their effects on blood composition one can judge well the health caring or toxic potential of antioxidants. In the present study, crude methanolic extracts containing antioxidants of some xerophytic herbs from the Cholistan desert of Pakistan were used to determine their in vitro effects on some hematological attributes like counts for granulocyte; leukocyte, lymphocyte; eosinophils; monocyte; granulocytes, Red Blood Cell, Mean Corpuscular, lymphocytes. Haemoglobin, Haemoglobin, Mean Corpuscular Haemoglobin, Concentration, Mean Corpuscular Volume, Packed Cell Volume, Hematocrit, and Red Cell Distribution. Data were analyzed statistically. Leucocytes, granulocytes, haemoglobin, and monocytes increased by extracts. Eosinophils, platelets, and RBC were decreased. There were recorded some exceptions. *Calotropis* stem decreased leucocytes, monocytes, and granulocytes. *Salsola* leaves decreased monocytes. Hemoglobin was increased by *Aerva* stem extract. Platelets were increased by *Haloxylon* stem extract.

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Introduction

Based on the findings of the recent years, the roles of antioxidants as health promoting factors are noticeable [1]. Medicinal plants play a pivotal role in the health care of ancient and modern cultures [2]. Antioxidant are inhibitors of oxidation, which prevents the oxidation and protect the cell from damage [3]. Antioxidants assist the body for adaptation to oxidative stress which causes cellular damage. The application of antioxidants or bioactive substances derived from medicinal plants presents a fundamental base for the development of therapeutic agents [4, 5]. These Naturally resourced molecules have been a source of an impressive number of medicines for thousands of years. Today as an estimation, more than two-thirds of the peoples of the world use plant resourced medicines [6]. A reasonable number of bodies of literature have reported the multiple pharmacological uses of bioactive compounds isolated from plants in the form of extracts [7]. These bioactive molecules in plant extracts are sources of many drugs [8].

Not only in the past but still in developing countries, these plants and their derived products have been used as a folk medicine for many diseases and ailment purposes [9]. The World Health Organisation (WHO) has reported that approximately 80% of the peoples in the whole world still use plants and their products for basic primary health needs [10] while these traditionally used medicines are available for 60% of the global peoples [9]. These derivatives of plants not only have been used in traditional systems of healing but still are the source of inspiration for several pharmaceutical medicines [11, 12]. In recent years, much attention has been paid to extract and analyze the biologically active substances from plants for their pharmacological nature [13,14].

Desert plants, due to the presence in a harsh environment, are considered a source of substantial bioactive compounds. Under unfavorable conditions, plants generate high concentrations of reactive oxygen species (ROS), causing oxidative stress to cells. To avoid these ROS generated stresses, plant cells synthesize antioxidants molecules of the enzymatic and non-enzymatic types [15,16]. Desert plants are the main source of antioxidants to be discovered which accumulate these

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bioactive compounds in their organs such as stem, leaves, roots, flowers, and seeds. These plants are usually used as a source of raw drugs. These raw drugs are used in traditional medicine and can be utilized as a source of modern medicines by pharmaceutical industries [17].

Of these bioactive ingredients, mostly are polyphenols. Polyphenols are bioactive antioxidants being beneficial for human health care. These reduce the clotting of blood and are used for the treatment of inflammatory diseases [18]. Phenolic compounds play a fundamental role in oxidative stress [19]. These phenolic compounds also have protective effects when present in plants used as food [20]. Flavonoid compounds such as Anastatins A and B and others are isolated from the desert plant are reported to have protective activities for hepatocytes [21]. The antioxidant potential of phenols in plant extracts has been reported effective in the prevention of cardiovascular disease and cancer [22-24]. Therefore, the studies for evaluation of the antioxidant potential of an extract of a different plant could contribute to revealing the values of these plant species as a source of new antioxidant compounds [25,26]. Different solvents are used for the extraction of specific bioactive plant ingredients [27]. It is reported that phenolic content is extracted more insolvent with higher polarity [28,29]. Methanol extract contains a high concentration of antioxidants as phenols [30-31] and has more ROS scavenging ability than the other solvent extracts [32-34]. Blood attributes are important in revealing the health status of an individual can be studied to evaluate the toxic or medicinal potentials of plant extracts after adding in it [35].

Materials and Methods

The aims of investigations

The investigation is aimed to explore the toxic or medicinal nature of methanol soluble antioxidants [36] of desert plants for in vitro changes in human blood. The selection of desert flora is based on their synthesis of antioxidants and their traditional use by local peoples for the treatment of some diseases. The choice of in vitro utilization of blood is based upon the determination of health status by blood parameters. Hence, the evaluation of hematological parameters might be a useful index employed for discrimination of toxic or medicinal potentials [35].

Experimental plan

Herbs from the Din Garh area of Cholistan desert were selected for antioxidants extraction. Methanol was used as a solvent for extraction procedure [37,38]. From a healthy volunteer, blood was taken for in vitro assessment after exposure to extract [39, 40]. Means of three replicates of each sample were compared with normal blood.

Climate and geology of the experimental site

The soil of the Cholistan desert of Pakistan is sandy and climate is hot [41]. Mean winter temperature ranges from 15 to 20°C and summer temperature from 35 to 50°C and, annual rainfall is less than 100 mm to 250 mm [42]. The vegetation of the Cholistan desert comprises xerophytic species that are adapted to these adverse conditions. The Cholistan desert is the world's seventh-largest desert. It covers an area of 26,000 sq. km., in the southern part of Punjab, Pakistan between 27° 42' and 29° 45' North and 69° 52' and 75° 24' East, [42] at an altitude of about 112m above sea level [43]. About 1.3 million ha of the Cholistan is comprised of dunes which are either stable or unstable [44].

Field survey and plant sample collections

After a preliminary survey and meetings with local peoples herbs were collected. Plants were identified by matching them with the labeled herbarium of the department (Dr.Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakarya University, Multan Pakistan, and the literature [45]. Specimens were collected according to an appropriate methodology [46,47] considering uniformity in age and size of plants and parts.

Crude herbal extract preparation

After washing with water and later with 2% methanol, specimens were dried at room temperature. Dried specimens were pulverized to a fine powder and crude Methanolic extract was prepared according to the method of Afolayan *et al.*,[48]. The Methanol used was of the highest purity. The extract was stored at -4°C.

Blood sampling and in vitro analysis

After approval from the BOS of the department, O⁺ blood was obtained from a healthy person volunteer of 25 years ago. The donor was selected after getting pieces of information about not taking any medications or addictive substances. Plant extract (1.0 ml) was added into 4ml blood (1:4) and was shaken smoothly. Blood without the addition of extract was taken as control. By using the Automated Hematology Analyzer machine he samples were analyzed for complete blood count tests (CBC).

Statistical analysis

The data for haematological indices were subjected to statistical analysis using Analysis Of Variance (ANOVA) at 5% level. Means were differentiated by Duncan's multiple range test [49]. Values were presented as Mean \pm SD.

Results

***Calligonum polygonoides* stem**

The marked increase was examined in HGB (28.55%); HCT (10.70%); MCV (14.21%); MCH (34.35%) and MCHC (22.10%) when compared with untreated group. The increase was statistically negligible for RDW (3.72%). The significant decrease was in leukocyte count (100%); lymphocyte (100%); monocyte (100%); eosinophils (100%); lymphocyte count (100%); platelets (2.16%) and MPV (13.85%). The increase was statistically negligible for granulocyte (100%); monocyte count (100%); eosinophils count (100%) and RBC (4.08%). No remarkable variation in granulocyte count was assessed by extract.

***Haloxylon salicornicum* leaves**

Methanolic extract act as a potent factor in determining the change in blood parameters. When compared with normal blood, it was found that the extract has played pivotal role in increasing the leukocyte count (457.73%); granulocyte (-3.00%); monocyte (1269.04%); granulocyte count (5720%); lymphocyte count (425%); monocyte count (6692.31%); HGB (11.69%); MCH (14.69%) and platelets (0.87%). Extract did not revealed statistically clear cut increase in MCV (5.14%); MCHC (16.19%) and RDW (13.48%). Methanolic extract role deviated from these expectations of promotion in lymphocyte, eosinophils, eosinophils count, RBC, HCT and MPV parameters. Extract showed a decrease in lymphocyte (8.47%); eosinophils (90.25%); MPV (9.52%). Extract did not revealed statistically clear cut decrease in eosinophils count (98.27%); RBC (5.82%) and HCT (0.35%).

Table 1: *In vitro* Assay for Phenolics rich Methanolic extract of shrubs from Cholistan desert in altering human haematological indices (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)	Lymphocyte %age (3.24)	Monocyte %age (1.26)	Eosinophils %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 A	0.68±0.03 ijklm	0.13±0.06 Gh	50.33±0.58 A
<i>Calligonum polygonoides</i> stem	0±0 m (100)	0±0 q (100)	0±0 p (100)	0±0 k (100)	0±0 e (100)	0±0 j (100)	0±0 n (100)	0±0 h (100)	0±0 f (100)
<i>Haloxylon salicornicum</i> leaves	31.4±2.32 a (-457.73)	28.43±1.40 kl (-21769.23)	43.87±1.70 i (8.47)	26.97±1.00 a (-1269.04)	0.27±0.06 bc (90.25)	8.73±0.76 a (-5720)	14.07±1.20 a (-425)	8.83±0.25 a (-6692.31)	0.87±0.21 e (98.27)
<i>Salsola imbricate</i> leaves	6.8±0.7 efghij (-20.78)	66.67±5.92 a (-51184.62)	32.4±2.33 no (32.36)	0±0 k (100)	0±0 e (100)	4.57±0.45 cde (-2946)	2.27±0.21 m (15.30)	0±0 h (100)	0±0 f (100)
<i>Salsola imbricata</i> root	8.8±0.75 d (-56.31)	35.87±1.15 ghi (-27492.31)	37.9±1.25 jkl (20.93)	26.23±2.36 ab (-1231.47)	0±0 e (100)	3.23±0.45 fg (-2053)	3.43±0.45 fghijk (-27.99)	2.4±0.46 b (-1746.15)	0.17±0.12 f (99.66)
<i>Salsola imbricata</i> stem	7.27±0.75 efg (-29.13)	54.33±2.35 d (-41692.31)	36.07±0.95 klm (24.74)	8.43±0.75 g (-327.92)	0±0 e (100)	4.1±0.66 cdef (-2633)	2.68±0.03 ijklm (0)	0.57±0.15 f (-338.46)	0.43±0.15 f (99.14)
<i>Calotropis procera</i> leaves	7.93±0.40 de (-40.85)	58.68±0.94 bc (-45038.46)	33.53±2.20 mno (30.04)	7.77±0.80 gh (-294.42)	0±0 e (100)	4.97±0.95 c (-3213)	2.68±0.03 ijklm (0)	0.5±0.1 fg (-284.62)	0.32±0.19 f (99.36)

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

***Salsola imbricate* leaves**

Methanolic extract substantially altered blood parameters. Extract showed a significant rise in leukocyte count (20.78%); granulocyte (51184.62%); granulocyte count (2946%); HGB (11.69%); MCH (14.69%) and platelets (0.87%). While some parameters MCV (5.14%); MCHC (16.19%) and RDW (13.48%) did not show an impressive response in this regard, and the increase was non-significant. But this was not consistent in all parameters. Extract showed a decrease in lymphocyte (8.47%); eosinophils (90.25%) and MPV (9.52%). While some parameters eosinophils count (98.27%); RBC (5.82%) and HCT (0.35%) did not show an impressive response in this regard and decrease was non-significant when compared with normal blood.

***Salsola imbricata* root**

Methanolic extract substantially altered blood parameters. Extract showed significant increase in leukocyte count (56.31%); granulocyte (64.11%); monocyte (1231.47%); granulocyte count (2053%); monocyte count (1746.15%); HCT (6.92%); MCH (11.59%); MPV (6.35%) by comparing with normal blood. Extract did not reveal statistically clear cut increase in

lymphocyte count (27.99%); HGB (8.05%); MCHC (6.58%) and RDW (2.26%). But this was not consistent in all parameters. The significant decrease was observed in lymphocyte (20.93%); eosinophils (100%); MCV (9.61%) and platelets (50.76%). Extract did not reveal statistically clear cut decrease in eosinophils count (99.66%) and RBC (2.91%) when compared with unrated group.

Table 1: (Continued..) *In vitro* Assay for Phenolics rich Methanolic extract of shrubs from Cholistan desert in altering human haematological indices (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
<i>Calligonum polygonoides</i> stem	4.94±0.07 abcdef (4.08)	10.67±0.4 a (-28.55)	25.13±0.65 efg (-10.70)	50.4±1.28 cdefghi (-14.21)	21.67±0.70 ab (-34.35)	42.7±1.6 a (-22.10)	14.23±0.45 cdefghi (-3.72)	452.33±44.61 c (2.16)	5.97±0.35 j (13.85)	18.57±0.45 c (-6.60)
<i>Haloxylon salicornicum</i> leaves	4.85±0.14 abcdef (5.82)	9.27±0.26 bcde (-11.69)	22.62±0.03 k (0.35)	46.40±0.56 jkl (-5.14)	18.50±0.46 efghi (-14.69)	40.63±0.76 abc (-16.19)	15.57±0.60 cdefg (-13.48)	466.33±39.40 c (-0.87)	6.27±0.25 ij (9.52)	18.03±0.25 cdefgh (-3.50)
<i>Salsola imbricata</i> leaves	5.01±0.10 abc (2.72)	9±0.25 defg (-8.43)	26.07±1.05 cde (-14.85)	51.37±2.31 bcdefgh (-16.41)	17.9±0.66 hij (-10.97)	34.8±0.31 d (0.49)	13.4±0.10 i (2.33)	288.33±17.56 defghi (37.63)	7.53±0.15 ab (-8.66)	17.07±0.15 jk (2.01)
<i>Salsola imbricata</i> root	5.00±0.06 abcd (2.91)	8.97±0.25 defgh (-8.05)	24.27±0.60 fghij (-6.92)	48.37±0.75 ghijk (9.61)	18±0.6 ghij (-11.59)	37.27±0.50abc de (-6.58)	14.03±0.15 efghi (-2.26)	227.67±29.74 i (50.76)	7.37±0.15 abcde (-6.35)	18.17±0.31 cdef (-4.30)
<i>Salsola imbricata</i> stem	4.92±0.13 abcdef (4.47)	8.83±1.52 efg (-6.39)	25.13±1.15 efg (-10.70)	51.27±2.30 bcdefgh (-16.18)	18.1±0.66 fghi (-12.21)	35.17±1.12 cde (0.57)	14.37±0.40 cdefgh (-4.74)	313.67±43.02 defgh (32.15)	6.92±0.03 efgh (0.14)	17.93±0.15 cdefghi (-2.93)
<i>Calotropis procera</i> leaves	5.14±0.01 a (0.19)	9.63±0.03 bcde (-14.46)	26.73±1.20 cd (-17.75)	52.17±1.36 bcde (-18.22)	18.13±0.32 fghi (-12.40)	35.73±1.21 cde (2.17)	14.43±0.71 cdefghi (-5.17)	337.67±87.50 de (26.96)	6.6±0.1 ghi (4.77)	18.2±0.3 cdef (-4.48)

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 Corpuscular Volume; MCH= Mean Corpuscular Haemoglobin; MCHC= Mean Corpuscular Haemoglobin concentration, RDW= Red Cell Distribution Width; Platelets= thrombocyte; MPV= Mean Platelet Volume

Salsola imbricata stem

Methanolic extract act as a potent factor in determining the change in blood parameters. The marked increase was showed in leukocyte count (29.13%); granulocyte (2633%); monocyte (327.92%); monocyte count (338.46%); HCT (10.70%); MCV (16.18%) and MCH (12.21%). The increase was statistically negligible for HGB (6.39%) and RDW (4.74%). Extract showed a decrease in lymphocyte (24.74%); eosinophils (100%) and platelets (32.15%). The decrease was statistically negligible for eosinophils count (99.14%); RBC (4.47%); MCHC (0.57%) and MPV (0.14%). The extract did not reveal any effect on Lymphocyte count by comparing it with the normal group.

Table 2: *In vitro* Assay for Phenolics rich Methanolic extract of shrubs from Cholistan desert in altering human haematological indices (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 10 ³ /μL (1.24)	Granulocyte %age (3.41)	Lymphocyte %age (3.24)	Monocyte %age (1.26)	Eosinophils %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 A	0.68±0.03 ijklm	0.13±0.06 gh	50.33±0.58 a
<i>Pseuda fruticosa</i> stem	31.4±2.33 a (-457.73)	28.43±1.41 kl (-21769.23)	43.87±1.70 i (8.47)	26.97±1.00 a (-1269.04)	0.27±0.06 bc (90.25)	8.73±0.76 a (-5720)	14.07±1.20 a (-425)	8.83±0.25 a (-6692.31)	0.87±0.21 e (98.27)
<i>Aerva javanica</i> flower	4.53 ±0.55 i (19.54)	10.23±0.75 p (-7769.231)	59.5 ± 1.4 f (-24.14)	25.23±1.12 b (-1180.71)	0.2±0.10 cd (92.78)	0.5±0.26 j (-233)	2.53±0.21 klm (5.60)	1.03±0.15 e (-692.31)	5.17±0.76 b (89.72)
<i>Aerva javanica</i> stem	6.4±0.95 gh (-13.68)	55.5±1.23 cd (-4592.31)	31.7±1.2 de (33.86)	12.13±1.31 e k (-515.74)	0±0 e (100)	3.9±0.75 def (-2500)	2.2±0.7 m (17.91)	0.77±0.15 ef (492.31)	0.32±0.13 f (99.36)
<i>Abutilon indicum</i> leaves	6.6±0.46 efghijk (-17.23)	59.6±1.05 b (-45746.15)	40.4±1.35 j (15.71)	0±0 k (100)	0±0 e (100)	3.93±0.80 def (-2520)	2.68±0.03 ijklm (0)	0±0 h (100)	0±0 f (100)
<i>Abutilon indicum</i> stem	6.47±0.50 efghijk (14.92)	33.2±1.21 hij (-25438.46)	67.1±1.15 c (-40.00)	0±0 k (100)	0±0 e (100)	2.13±0.35 h (-1320)	4.37±0.60 def (63.06)	0±0 h (100)	0±0 f (100)

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

Calotropis procera leaves

Different sensitivity range was found in response of blood parameters treated with Methanolic extract. Mean values for enhancing role of Methanolic extract were as for leukocyte count (40.85%); granulocyte (3213%); monocyte (294.42%); granulocyte count (497%); HGB (14.46%); HCT (17.75%); MCV (18.22%); MCH (12.40%) and platelets (26.96%). While some parameters as monocyte count (284.62%); RBC (0.19%); MCHC (2.17%) and MPV (4.77%) did not reveal impressive response in this regard and increase was non-significant. While some parameters showed opposite index of values. Extract showed a decreased in lymphocyte (30.04%); eosinophils (100%) and platelets (26.96%). While some parameters as eosinophils count (99.36%); RBC (0.19%); MCHC (2.17%); MPV (4.77%) did not show impressive response in this regard and increase was non-significant. Extract failed to change lymphocyte count.

Pseuda fruticosa stem

The stimulating behavior of Methanolic extract varied considerably. Mean values for the enhancing role of Methanolic extract were leukocyte count (457.73%); granulocyte (2.55%); monocyte (1269.04%); granulocyte count (5720%); lymphocyte count (425%); monocyte count

Table 2: (Continued..) *In vitro* Assay for Phenolics rich Methanolic extract of shrubs from Cholistan desert in altering human haematological indices (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
<i>Pseuda fruticosa</i> stem	4.85±0.14 abcdef (5.83)	9.27±0.40 bcde (-11.28)	22.62±0.03 k (0.48)	46.4±0.56 jkl (-5.14)	18.5±0.46 efghi (-14.69)	40.63±1.60 abc (-16.19)	14.57±0.60 cdefg (-6.20)	466.33±39.40 c (27.40)	6.27±0.25 ij (9.52)	18.03±0.01 cdefgh (-3.50)
<i>Aerva javanica</i> flower	5.02±0.11 abc (2.52)	7.93±0.38 i (4.80)	23.4±0.5 hijk (-2.94)	45.5±0.5 kl (-3.10)	15.63±0.85 m (3.10)	33.13±0.25 e (5.26)	14.43±0.50 cdefg (-5.17)	233.33±37.52 hi (63.67)	7.7±0.56 a (-11.11)	16.5±0.01 k (5.28)
<i>Aervajavanica</i> stem	4.85±0.25 abcdef (5.83)	9.03±0.35 defg (-8.40)	23.8±0.75 ghijk (-4.70)	47.97±1.05 ijk (-8.70)	18.49±0.91 efghi (-14.63)	38.17±1.05a bcde (-9.15)	14.23±0.25 cdefghi (-3.71)	319±39.96 defg (50.34)	7.07±0.40 cdefg (-2.02)	17.3±0.06 ij (0.69)
<i>Abutilon indicum</i> leaves	4.85±0.13 abcdef (5.83)	9.41±0.14 defg (-12.97)	28.23±0.75 b (-24.20)	57.83±0.95 a (-31.04)	19.33±0.65 defg (-19.84)	33.4±0.66 e (4.49)	14.8±0.46 cdef (-7.87)	264.67±40.0 efghi (58.80)	6.88±0.03 fg (0.72)	17.67±0.1 fghi (-1.44)
<i>Abutilon indicum</i> stem	5.15±0.01 a (0)	9.53±0.25 bcde (-14.41)	27.1±0.66 bc (-19.23)	52.3±0.75 bcd (-18.51)	18.4±0.6 efghi (14.07)	35.27±0.50 cde (-0.86)	14.27±0.31 cdefghi (-4.01)	315.67±44.12 defg (50.86)	6.88±0.03 fgh (0.72)	17.83±0.01 efghi (-2.35)

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 Corpuscular Volume; MCH= Mean CorpuscularHaemoglobin; MCHC= Mean CorpuscularHaemoglobin concentration, RDW= Red Cell Distribution Width; Platelets= thrombocyte; MPV= Mean Platelet Volume

(6692.31%); HGB (11.28%); MCH (14.69%); platelets (27.40%) and MPV (9.52%). While some parameters did not showed impressive response in this regard and increase was non-significant as MCV (5.14%); MCHC (16.19%) and RDW (6.20%). Methanolic extract role deviated from these expectations of increment in some of the parameters. Extract showed a decrease in lymphocyte (8.47%); eosinophils (90.25%); eosinophils count (98.27%); platelets (27.40%) and MPV (9.52%). Non significant reduction has been observed in RBC (5.83%) and HCT (0.48%) as compared to the normal blood.

Aerva javanica flower

Different sensitivity range was found in response of blood parameters treated with Methanolic extract. The application of extract seems promising in enhancing the granulocyte (94.44%); lymphocyte (24.14%); monocyte (1180.71%); monocyte count (692.31%) and MPV (11.11%). Although not statistically justified, but to a substantial level of increase, a 2.94%, 3.10%, 233% and 5.17% rise in HCT, MCV, granulocyte count and RDW respectively was observed when extract was applied. The increasing augmentation could not have pace for some parameters. Extract showed a decrease in eosinophils (92.78%) and platelets (63.67%). Extract did not reveal statistically clear cut decrease in leukocyte count (19.54%); lymphocyte count (5.60%); RBC (2.52%); HGB (4.80%); MCH (3.10%) and MCHC (2.56%) as compared to the normal blood.

Aerva javanica stem

An data of percentage differences revealed increase of blood parameter by Methanolic extract application. Exposure to extract has strong impact on leukocyte count (13.68%); granulocyte (56.67%); monocyte (515.74%); granulocyte count

(2500%); MCV (8.70%) and MCH (14.63%). The increase was statistically negligible for HGB (8.40%); HCT (4.70%); MCHC (9.15%); RDW (3.71%). But the extract failed to maintain the trend in some of the parameters. The maximum decrease regarding role of extract was for lymphocyte (33.86%); eosinophils (100%); monocyte count (492.31%); eosinophils count (99.36%) and platelets (58.80%). The increase was statistically negligible for lymphocyte count (17.91%) and RBC (5.83%) as compared to the control group.

***Abutilon indicum* leaves**

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 (45746.15%); granulocyte count (56.33%); HGB (12.97%); HCT (24.20%); MCV (31.04%); MCH (19.84%) and RDW (7.87%). Extract did not reveal statistically clear cut increase in leukocyte count (17.23%). Methanolic extract role deviated from these expectations of promotion in some parameters. Extract showed a decrease in lymphocyte (15.71%); monocyte (100%); eosinophils count (100%); platelets (58.80%) and MPV (0.72%). Extract revealed no significant difference in monocyte count (100%); RBC (5.83%); MCHC (4.49%); RDW (%); platelets (%); MPV (%). Extract revealed no effect on lymphocyte count shown by comparing the normal blood.

***Abutilon indicum* stem**

Methanolic extract proved its significant influence when applied. Mean values were as for granulocyte (25438.46%); lymphocyte (40.00%); granulocyte count (1320%); HGB (14.41%); HCT (19.23%); and MCV (18.51%). While some parameters did not show impressive response in this regard and increase was non-significant as MCHC (0.86%) and RDW (4.01%). An exception in this relationship was found in some parameters. Blood parameters monocyte (100%); eosinophils (100%); lymphocyte count (63.06%); eosinophils count (100%); MCH (14.07%) and platelets (50.86%) revealed a significant decreased when treated with extract. While some parameters did not showed impressive response in this regard and decrease was non-significant as leukocyte count (14.92%); monocyte count (100%) and MPV (0.72%). Extract failed to change RBC as compared to the control group.

Discussion

The plant extracts containing antioxidants such as phenolics protects human against diseases. [50]. Blood parameters are diagnostic for physiological status of an organism [51]. Addition of antioxidants extract in blood changes the blood characteristics due to free radical scavenging activity [52]; anti-glycosylation [53]; thrombolytic potential [40], anticoagulation by plant extract [39] or by genotoxicity [54]. A hematological disorders like low haemoglobin contents causes anaemia [55]. Low levels of hemoglobin and RBC can be due to iron deficiency or blood cell destruction which causes also anemia [56]. Massive hemolysis increases the levels of free hemoglobin in blood and causes hemoglobinemia [57]. High MCV mean larger RBC size.. Mean corpuscular hemoglobin (MCH) is average amount of hemoglobin in single red blood cell. Increase in MCH is due to anemias [58]. Mean corpuscular hemoglobin concentration (MCHC) is for average concentration of hemoglobin in a single red blood cell. A low MCHC is iron deficiency or indication 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. PCV (called Packed Cell Volume) is used to signal known or suspected anemia [59]. Mean cell/corpuscular volume (MCV) is size or volume of a red blood cell. Low MCV is due to iron deficiency [60].

Our results revealed a different action of Methanolic extracts sourced from different plants parts on various hematological attributes. The results are in accordance to the findings of Straus [61] regarding effects on RBC and Hb concentration (MCH, MCHC) and contradict to the findings of Loharet *al.* [62]. It has been reported that plants extracts vary in their effects and are non-specific in their actions [63]. Antioxidants are synthesized in response to stresses for neutralizing the reactive oxygen species (ROS). In blood damage to cells and biomolecules caused by reactive oxygen species (ROS) is nullified by antioxidant [64]. The concentration ratio of antioxidant to ROS might play role against the hemolytic activity of ROS by stabilizing blood cells and molecules or by their direct action on ROS.

Erythrocytes due to having high concentrations of polyunsaturated fatty acids in their membranes are considered as major target of free radicals [65]. 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/extract can have more effect on erythrocytes than any other blood cells [66]. Results of present studies revealed that Methanolic extracts of desert plants specimens reduced the RBC. The hemolysis of red blood cells by ROS releases hemoglobin from these cells.

Results revealed that Methanolic extracts of desert plant specimens 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 [67]. Mean cell volume (MCV) is the volume of red cells. An increase in MCV with a decrease in MCHC accounts for reduced osmotic fragility of membrane [68]. MCV, MCH and MCHC relates to individual red blood cells while Hb and RBC are concerned with the total numbers of red blood cells [69]. The MCV determines the size of the RBCs. High MCV mean the RBCs will be larger and are termed as macrocytic. When the MCV is low, the RBCs are smaller called as microcytic. RBCs of normal size are termed normocytic. These categories of size are used to classify anemias. A significant reduction in MCV,

when observed, is due to interference by hemoglobin in iron uptake. Furthermore, it is reported that decrease in the blood cells may be due to the increased glycosylation of membrane proteins which can cause hyperglycemia.

Methanolic extracts of some specimens reduced the haemoglobin while others induced an increase. This change in haemoglobin quantity in blood might be due to Iron deficiency as Iron is a part of heme group of hemoglobin. The Iron deficiency could be due to interference of plant extract biomolecules with iron directly or interference during its biosynthesis metabolism. A failure in hemoglobin production results in smaller. This causes anemia and thalassemia. Iron deficiency might be due to desert plant ROS which cause mobilization of Fe^{2+} by Ca^{2+} via Fenton reaction [70]. The increase in Hb (MCH and MCHC) facilitates oxygen transportation 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 [62].

Reduction in platelets was observed by Methanolic extracts. Similarly, if there is decline in platelet, it results in myeloma and lymphoma [71, 72]. Platelet change might be by their adhesion to collagen and platelet aggregation by ROS species of desert plants [73]. Plant antioxidants might change blood platelet by antiplatelet activity [74]. Blood platelets reduction may be beneficial as platelets decrease the blood viscosity which adds positive contribution to blood pressure and also may be beneficial by view of the clinical haematology.

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