



www.ajbrui.net

Afr. J. Biomed. Res. Vol.15 (January 2012); 41 - 47

Research Article

Effects of Crude Extracts of *Portulaca oleracea* on Haematological and Biochemical Parameters in Albino Rats

¹Oyedeji K.O. and ²Bolarinwa A.F

¹Department of Physiology, Ladoko Akintola University of Technology, Ogbomosho, Nigeria.

²Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT: The effects of oral administration of aqueous (AEPO) and methanolic (MEPO) extracts of *Portulaca oleracea* at various doses (25mg/kg BW, 50mg/kgBW and 75mg/kgBW) on haematological and plasma biochemical parameters of albino rats were investigated. The extracts were administered on daily basis for 30 days and blood samples for analyses were collected on days 15 and 30. Rats treated with 25mg/kg BW AEPO for 15 days showed a significant ($P<0.05$) decrease in WBC and neutrophil counts and a significant increase in lymphocyte counts. Treatment of rats with 25mg/kg BW AEPO for 15 days caused significant ($P<0.05$) decrease and increase in the neutrophil and lymphocyte counts respectively. Treatment with 25mg/kg MEPO for 15 days caused significant ($P<0.05$) increase in both the MCV and MCH. However, the administration of AEPO and MEPO at all the treatment doses for 30 days produced no significant ($P>0.05$) change in all the haematological parameters. Treatment of rats with 25mg/kg AEPO for 15 days caused significant ($P<0.05$) increase and decrease in the albumin and globulin levels respectively. There were significant reductions in the values of total protein, albumin and globulin of rats treated with 25mg/kg BW of AEPO and 75mg/kg BW of MEPO at 30 days treatment. The administration of all the treatment doses for 15 and 30 produced no significant ($P>0.05$) change in the activities of AST and ALT. These findings on haematological and biochemical parameters suggest that the possible changes in blood chemistry of the treated rats were due to the extracts of *Portulaca oleracea*.

Key words: *Portulaca oleracea*, haematological parameters, biochemical parameters, albino rats

INTRODUCTION

Portulaca oleracea (Family Portulacaceae) is a warm-climate annual herb and has a cosmopolitan distribution. It is commonly called purslane in English language and esan omode or papasan in Yoruba language (Burkill, 1997). It is used medicinally in Ghana for heart-palpitations (Johnson, 1997). The plant is used as a diuretic in Nigeria (Ainslie, 1937). A tisane of the plant is drunk in Trinidad as a vermifuge (Wong, 1976).

It has been reported that aqueous and methanolic extracts of *P. oleracea* have contractile effects on isolated intestinal smooth muscle in in-vitro preparations (Oyedeji *et al.*, 2007). Recent studies have also shown that extracts of *P. oleracea* cause reduction in locomotion activity and an increase in the onset time of pentylenetetrazole (PTZ) induced convulsion in rats (Radhakrishnan *et al.*, 2001).

This study was aimed at investigating the effects of aqueous and methanolic extracts of *Portulaca oleracea* on the haematological and plasma biochemical parameters of albino rats.

MATERIALS AND METHODS

Experimental Animals

Adult male and female albino rats weighing between 150g and 250g bred in the Pre-clinical Animal House of the college of Medicine, University of Ibadan were used. They were housed under standard laboratory

*Correspondence: (Email): sinaoyedeji@yahoo.com

conditions with a 12 hours daylight cycle and had free access to feed and water; and were acclimatized to laboratory conditions for two weeks before the commencement of the experiments.

Plant Material

Fresh specimens of *Portulaca oleracea* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan. It was identified and assigned a voucher specimen number FHI 108334 in FRIN.

Preparation of the Extracts

Large quantity (2kg) of the fresh specimens of *Portulaca oleracea* were washed free of soil and debris, and the roots were separated from the leaves and stems. The leaves and stems were air-dried for six weeks and then pulverished using laboratory mortar and pestle and was later divided into two samples A and B.

(i) Aqueous Extract of *Portulaca oleracea* (AEPO): Weighted Portions (431.33g) of sample A were macerated and extracted with distilled water (1:2 wt/vol) for 72 hours at room temperature (26 – 28°C). The resulting solution was then filtered using a wire-gazed and a sieve with tiny pores (0.25mm). The distilled water was later evaporated using steam bath to give a percentage yield of 11.8% of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

(ii) Methanolic Extract of *Portulaca oleracea* (MEPO): Weighted portions (420.52g) of sample B were macerated and extracted with 70% methanol (1:2 wt/vol) for 72 hours at room temperature (26 – 28°C). The resulting solution was then filtered using a wire-gauze and a sieve with tiny pores (0.25mm). The 70% methanol was later evaporated using steam bath to give a percentage yield of 10.2% of the starting material. The dried material was reconstituted in distilled water to make up test solutions of known concentrations.

Ten grams of AEPO and MEPO were dissolved in 100ml of distilled water to give a concentration of 0.1g/ml. The dosages of AEPO and MEPO administered in these studies were in accordance with those reported by (Miladi-Gorgi *et al.*, 2004).

Animal Grouping and Extracts Administration

Thirty-five male albino rats weighing between 150g-200g were randomly divided into seven groups, with each group consisting of five animals.

The seven groups of rats were subjected to the following oral treatments once a day for thirty days and

blood samples were collected for analysis on day 15 and on day 30 at the end of the experiment: Group I received 25mg/kg BW of AEPO
Group II received 50mg/kg BW of AEPO
Group III received 75mg/kg BW of AEPO
Group IV received 25mg/kg BW of MEPO
Group V received 50mg/kg BW of MEPO
Group VI received 75mg/kg BW of MEPO
Group VII received 0.5ml of distilled water as the control group.

Collection of Blood samples

Blood used for haematological and plasma biochemical studies was collected from diethyl ether anaesthetized rats into Lithium-heparinized bottles using sino-ocular puncture method. The blood samples were centrifuged for 5 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determinations of the biochemical parameters.

Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) were evaluated using the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined using the cyanomethaemoglobin method (Jain, 1986), and the packed cell volume (PCV) was determined by the micro-haematocrit method (Dacie and Lewis, 1991). Schilling method of differential leukocyte counts was used to determine the distribution of the various white blood cells (Mitruka and Rawnsley, 1977). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed using the method of Jain (1986).

Determination of Plasma Biochemical Parameters

The total protein concentration was determined using the Biuret method (Reinhold, 1953) and the albumin concentration by the method of Doumas *et al.*, (1971). The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined using the method of Duncan *et al.*, (1994).

STATISTICAL ANALYSIS

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least

significant difference (LSD). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Effects on haematological parameters

The effects of AEPO and MEPO at various doses on the haematological parameters of albino rats on days 15 and 30 are shown in Tables 1 and 2 respectively.

Administration of AEPO and MEPO at all doses for 15 days produced no significant change ($P > 0.05$) on the PCV relative to the control. Also, rats treated with 25mg/kg BW AEPO for 15 days showed a significant decrease ($P < 0.05$) in WBC relative to the control. Treatment of rats with 25mg/kg BW AEPO and 50mg/kg BW AEPO for 15 days produced significant ($P < 0.05$) reductions in the neutrophil counts relative to the control. The administration of 25mg/kg BW AEPO and 50mg/kg BW AEPO for 15 days produced

significant ($P < 0.05$) increase in the lymphocyte counts relative to the control. Treatment of rats with 25mg/kg BW AEPO for 15 days produced no significant ($P > 0.05$) change in the platelet counts relative to the control. Treatment of rats with 75mg/kg BW AEPO, 25mg/kg BW MEPO and 75mg/kg BW MEPO for 15 days produced no significant ($P > 0.05$) change in the eosinophil counts relative to the control. The administration of 25mg/kg BW MEPO for 15 days produced significant ($P < 0.05$) increase in both MCV and MCH relative to their respective controls. The administration of AEPO and MEPO at all the treatment doses for 15 days produced no significant ($P > 0.05$) changes in the haemoglobin (Hb) concentration, MCHC, RBC and monocytes counts relative to their respective controls. The administration of AEPO and MEPO at all the treatment doses for 30 days produced no significant ($P > 0.05$) changes in all the haematological parameters relative to their controls..

Table 1:
Effects of AEPO and MEPO on haematological parameters of rats on day 15 (n=5)

Parameters	Control	25mg/kg BW AEPO	50mg/kg BW AEPO	75mg/kg BW AEPO	25mg/kg BW MEPO	50mg/kg BW MEPO	75mg/kg BW MEPO
PCV (%)	42.33 ± 1.20	41.40 ± 1.25	42.50 ± 0.87	43.40 ± 1.08	41.40 ± 2.02	41.00 ± 1.41	41.00 ± 2.80
Hb (g/dl)	13.63 ± 0.50	13.36 ± 0.49	13.70 ± 0.34	14.06 ± 0.44	13.38 ± 0.68	13.18 ± 0.49	13.25 ± 0.94
RBC (x10 ⁶ /μL)	7.35 ± 0.26	6.90 ± 0.17	7.10 ± 0.11	7.28 ± 0.12	6.70 ± 0.43	6.97 ± 0.29	6.86 ± 0.52
MCV (FL)	57.60 ± 0.52	60.16 ± 0.75	59.87 ± 0.46	59.62 ± 0.72	62.15 ± 1.61*	58.97 ± 1.80	59.94 ± 1.51
MCHC (g/dl)	32.19 ± 0.29	32.25 ± 0.22	32.23 ± 0.16	32.38 ± 0.23	32.32 ± 0.30	32.13 ± 0.09	32.30 ± 0.19
MCH (pg)	18.54 ± 0.17	19.34 ± 0.38	19.30 ± 0.24	19.30 ± 0.33	20.09 ± 0.59*	18.95 ± 0.60	19.36 ± 0.51
WBC (x10 ³ /μL)	9.57 ± 0.31	6.51 ± 0.44*	11.31 ± 0.82	10.98 ± 0.54	11.20 ± 0.58	11.76 ± 1.04	10.74 ± 0.78
Platelets (x10 ⁵ /μL)	1.35 ± 0.06	1.07 ± 0.04	1.18 ± 0.04	1.34 ± 0.32	1.18 ± 0.05	1.17 ± 0.05	1.15 ± 0.07
Neutrophils (%)	34.00 ± 2.65	26.20 ± 1.66*	27.75 ± 1.38*	31.20 ± 1.80	30.40 ± 1.69	29.20 ± 0.80	31.50 ± 2.75
Lymphocytes (%)	63.00 ± 3.21	69.20 ± 0.80*	69.50 ± 1.50*	65.60 ± 1.91	66.00 ± 1.95	68.40 ± 0.87	65.00 ± 3.24
Eosinophils (%)	1.33 ± 0.33	1.75 ± 0.48	1.25 ± 0.25	1.67 ± 0.33	2.00 ± 0.45	1.25 ± 0.25	1.75 ± 0.48
Monocytes (%)	1.67 ± 0.33	1.60 ± 0.40	1.50 ± 0.29	2.20 ± 0.37	1.60 ± 0.40	1.75 ± 0.48	1.75 ± 0.48

Asterisks indicate significant difference from control at $P < 0.05$

Table 2:

Effects of AEPO and MEPO on haematological parameters of rats on Day 30 (n=5)

Parameters	Control	25mg/kg AEPO	50mg/kg BW AEPO	75mg/kg AEPO	25mg/kg BW MEPO	50mg/kg MEPO	75mg/kg BW MEPO
PCV (%)	43.33 ± 0.67	40.25 ± 3.01	44.25 ± 1.75	44.40 ± 1.17	44.80 ± 1.50	41.75 ± 1.03	43.25 ± 1.70
Hb (g/dl)	14.07 ± 0.24	12.08 ± 0.47	14.25 ± 0.68	14.46 ± 0.47	14.72 ± 0.60	13.45 ± 0.27	13.93 ± 0.65
RBC (x10 ⁶ / μL)	7.58 ± 0.03	6.95 ± 0.65	7.43 ± 0.26	7.59 ± 0.37	7.74 ± 0.32	7.22 ± 0.20	7.66 ± 0.41
MCV (FL)	57.17 ± 0.92	58.17 ± 1.07	59.53 ± 1.10	58.91 ± 2.78	57.99 ± 1.01	57.86 ± 1.26	56.64 ± 1.11
MCHC (g/dl)	32.46 ± 0.14	30.29 ± 1.42	32.18 ± 0.28	32.55 ± 0.24	32.82 ± 0.30	32.24 ± 0.51	32.17 ± 0.27
MCH (pg)	18.56 ± 0.31	17.66 ± 1.12	19.16 ± 0.47	19.19 ± 1.00	19.03 ± 0.32	18.67 ± 0.64	18.22 ± 0.35
WBC (x10 ³ / μL)	9.73 ± 0.57	7.98 ± 0.64	6.68 ± 0.74	7.68 ± 0.29	7.47 ± 0.58	8.31 ± 0.58	8.78 ± 1.15
Platelets (x10 ⁵ / μL)	1.15 ± 0.81	1.11 ± 1.03	1.02 ± 0.86	1.06 ± 0.83	1.20 ± 1.06	1.35 ± 1.04	1.32 ± 0.98
Neutrophils (%)	33.00 ± 2.31	32.50 ± 1.44	34.00 ± 1.96	33.80 ± 2.08	32.20 ± 2.08	36.50 ± 1.85	32.25 ± 2.14
Lymphocytes (%)	64.67 ± 2.03	64.25 ± 1.11	62.00 ± 2.00	64.80 ± 1.59	64.20 ± 2.06	60.25 ± 1.38	64.25 ± 1.65
Eosinophils (%)	1.00 ± 0.00	1.50 ± 0.29	1.75 ± 0.48	1.60 ± 0.40	1.80 ± 0.37	1.75 ± 0.48	1.75 ± 0.48
Monocytes (%)	1.33 ± 0.33	1.75 ± 0.48	1.50 ± 0.29	1.80 ± 0.37	1.80 ± 0.37	1.50 ± 0.29	1.75 ± 0.48

Asterisks indicate significant difference from control at $P < 0.05$ **Effects on plasma biochemical parameters**

The effects of AEPO and MEPO at various doses on the plasma biochemical parameters of albino rats on days 15 and 30 of the experiment are shown in Tables 3 and 4 respectively.

The administration of all the treatment doses of AEPO and MEPO for 15 days produced no significant ($P > 0.05$) change in the total protein levels relative to their controls. However, treatment of rats with 25mg/kg BW AEPO and 75mg/kg BW MEPO for 30 days produced significant ($P < 0.05$) decrease in the total protein levels relative to the control. Treatment of rats with 25mg/kg BW AEPO for 15 days produced a significant ($P < 0.05$) increase in the albumin level relative to the control. However, treatment of rats with 25mg/kg BW AEPO for 30 days produced a significant ($P < 0.05$) decrease in the albumin level relative to the control. Treatment of rats with 25mg/kg BW AEPO and 75mg/kg BW MEPO for 15 and 30 days respectively produced significant ($P < 0.05$) decrease in the globulin levels relative to their controls. Treatment with all the doses of AEPO and MEPO for 15 days

produced no significant ($P > 0.05$) change in the activities of AST and ALT relative to their respective controls. Likewise, treatment with all the doses of AEPO and MEPO for 30 days produced no significant ($P > 0.05$) change in the activities of AST and ALT relative to their controls.

DISCUSSION

The values obtained for RBC showed the no-significant effects of 30-day treatment of rats with AEPO and MEPO on red blood cells (RBC) counts and indices relating to it (Hb, PCV, MCV, MCH and MCHC) when compared with the control. This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis). This also shows that the AEPO and MEPO do not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996).

Table 3:

Effects of AEPO and MEPO on the plasma biochemical parameters of rats on day 15 (n=5)

Parameters	Control	25mg/kg	50mg/kg	75mg/kg	25mg/kg	50mg/kg	75mg/kg
		BW AEPO	BW AEPO	BW AEPO	BW MEPO	BW MEPO	BW MEPO
Total protein (gm%)	4.70 ± 0.30	4.30 ± 0.30	5.10 ± 0.10	4.35 ± 0.05	4.85 ± 0.05	4.60 ± 0.00	4.50 ± 0.20
Albumin (gm%)	1.75 ± 0.15	2.35 ± 0.05*	2.05 ± 0.25	2.10 ± 0.20	2.00 ± 0.20	1.90 ± 0.20	1.95 ± 0.05
Globulin (gm%)	2.95 ± 0.45	1.95 ± 0.25*	3.05 ± 0.15	2.25 ± 0.15	2.85 ± 0.25	2.70 ± 0.20	2.55 ± 0.15
AST (μ/L)	9.50 ± 2.50	14.50 ± 1.50	23.00 ± 0.00	18.50 ± 8.50	23.00 ± 4.00	13.00 ± 3.00	18.00 ± 5.00
ALT (μ/L)	6.00 ± 2.00	12.00 ± 5.00	23.00 ± 11.00	19.00 ± 15.00	27.00 ± 2.00	13.50 ± 7.50	19.00 ± 2.00

Asterisks indicate significant difference from control at $P < 0.05$ **Table 4:**

Effects of AEPO and MEPO on the plasma biochemical parameters of rats on day 30 (n=5)

Parameters	Control	25mg/kg	50mg/kg	75mg/kg	25mg/kg	50mg/kg	75mg/kg
		BW AEPO	BW AEPO	BW AEPO	BW MEPO	BW MEPO	BW MEPO
Total protein (gm%)	6.63 ± 0.12	5.55 ± 0.75*	6.40 ± 0.23	6.40 ± 0.20	6.27 ± 0.39	5.73 ± 0.15	5.60 ± 0.40*
Albumin (gm%)	3.97 ± 0.23	2.70 ± 0.20*	4.03 ± 0.22	3.67 ± 0.29	3.43 ± 0.43	3.60 ± 0.31	4.10 ± 0.50
Globulin (gm%)	2.67 ± 0.19	2.85 ± 0.55	2.17 ± 0.33	2.80 ± 0.20	2.83 ± 0.15	2.13 ± 0.20	1.50 ± 0.10*
AST (μ/L)	19.33 ± 1.86	12.00 ± 7.00	21.00 ± 0.58	15.67 ± 4.70	22.67 ± 2.96	12.13 ± 4.44	12.50 ± 6.50
ALT (μ/L)	26.67 ± 2.85	10.00 ± 7.00	25.33 ± 0.33	20.67 ± 5.60	31.00 ± 1.73	13.67 ± 6.89	16.00 ± 11.00

Asterisks indicate significant difference from control at $P < 0.05$.

The non-significant effects of the 30-day treatment with AEPO and MEPO at all doses also indicate that there were no change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and haemoglobin (Hb) are very important in transferring respiratory gases (De Gruchy, 1976). It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia (American Diabetes Association, 2000), thus, the 30-day treatment with AEPO and MEPO may not have the potential to induce anemia or polycythemia. Also, the 30-day treatment with AEPO and MEPO at all treatments may not have adverse effects on the bone marrow, kidney and haemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism (Young and Maciejewski, 1997). The non-significant changes in the total white blood cell, neutrophil, eosinophil, monocyte and lymphocyte counts after 30-day treatment with AEPO and MEPO at all the treatment doses suggest that the immune system has not been compromised. The insignificant change in the value of platelet after the 30-day treatment with

AEPO and MEPO is also an indication that the extracts do not have the potential to stimulate thrombopoietin production (Li *et al.*, 1999). However, the 15-day treatment of rats with MEPO (25mg/kg) cause a significant increase in the MCV and MCH, this indicates that MEPO (25mg/kg) could induce macrocytic anaemia since increased MCV and MCH are known to be indicative of macrocytic anaemia. The significant increase in the MCV could also be due to methanol which is the extractive medium, since it has been reported that an elevated MCV is associated with alcoholism (Tonnesen, *et al.*, 1986).

Also, the significant decrease in the total white blood cell counts after the 15-day treatment of rats with AEPO (25mg/kg) may imply a reduction in the ability of the body to respond to infections. It has been reported that toxic substances caused decrease in total white blood cell counts through either bone marrow depression or competition with folic acid utilization to cause leucopenia (Jain, 1986); this may implies that AEPO (25mg/kg BW) is toxic to leucocytes on short-term basis which may be mediated through any of the aforementioned mechanisms, this toxicity might be due to saponin which is one of the phytochemical constituents of *Portulaca oleracea* which has been

reported to cause life-threatening animal toxicity (Cornell, 2008).

The plasma biochemical study shows that 30-day treatment of rats with AEPO (25mg/kg BW) and MEPO (75mg/kg BW) produce significant decrease in the total plasma protein levels which might indicate a reduction in the buffering capacity of the blood as well as a decrease in colloid osmotic pressure which may lead to the loss of fluid from the capillaries, since plasma proteins have been reported to be responsible for 15% of the buffering capacity of blood (Ganong, 2005) and that osmotic pressure caused by the plasma proteins (called colloid osmotic pressure) tends to cause fluid movement by osmosis from the interstitial spaces into the blood (Guyton and Hall, 2006). However, short-term treatment with all the doses of AEPO and MEPO produced no significant changes in the total plasma protein levels which might indicate that the buffering capacity of the blood and the body fluid balance have not been compromised. The significant decrease and increase in the albumin levels obtained after 30-day and 15-day treatment respectively of rats with AEPO (25mg/kg BW) might indicate a decrease and an increase respectively in plasma levels of metals, ions, fatty acids, amino acids, bilirubin and enzymes; since it has been reported that albumin serves as a carrier for metals, ions, fatty acids, amino acids, bilirubin, enzymes, and drugs (Ganong, 2005). The significant decrease in globulin level obtained after 30-day treatment of rats with MEPO (75mg/kg BW) might indicate a compromise in both the natural and acquired immunity of the body against invading organisms. Report has shown that globulins are principally responsible for the body's both natural and acquired immunity against invading organisms (Guyton and Hall, 2006).

The 30-day and 15-day treatments of rats with AEPO and MEPO at all treatments produced no significant change in the activity of ALT which indicates absence of hepatic lesion in the treated rats. Presence of ALT in the liver and other cells has been reported to be particularly useful in measuring hepatic necrosis, especially in small animals (Duncan *et al.*, 1995). Also, the 30-day and 15-day treatments of rats with AEPO and MEPO at all the treatment doses produced no significant changes in the activity of AST which may indicate absence of tissue necrosis induction by these extracts; it has been reported that elevation in the activity of AST can be associated with cell necrosis of many tissues; pathology involving the skeletal or cardiac muscle and/or hepatic parenchyma, allows leakage of large amounts of this enzyme into the blood (Bush, 1991).

In conclusion, this study has shown that the crude extracts of *Portulaca oleracea* could have some toxic and beneficial potentials on the blood chemistry of albino rats. However, the effects of crude extracts of this plant on human blood chemistry are unknown; nevertheless, considering these findings in animal model, it is recommended that caution should be exercised in the consumption of *Portulaca oleracea*.

REFERENCES

- Ainslie J.R. (1937):** The list of plants used in native medicine in Nigeria, Imp. Forest. Inst. Oxford Inst. Paper 7(mimeo).
- American Diabetes Association (2000):** Nutrition recommendation and principles for people with diabetes mellitus clinical practice recommendations *Diabetes care* 23:543-6.
- Burkill H.M. (1997):** The useful plants of West Tropical Africa, vol.4. The Whitefriars Press Limited, Tonbridge, Kent TN9 1QR, Great Britain.
- Bush B.M. (1991):** Interpretations of Laboratory Results for Small Animal Clinicians. Blackwell Scientific Publications, London.
- Cornell, op cit, MetaCyc (2008):** Saponin biosynthesis. www.biocyc.org/META/NEW-IMAGE.com.
- Dacie J.V., Lewis S.M. (1991):** Practical haematology, 7th edition ELBS with Churchill Livingstone, England, pp 37-85.
- De Gruchy G.C. (1976):** Clinical haematology in Medical Practice. Blackwell Scientific Publication. Oxford, London pp. 33-57.
- Doumas B.T., Watson W., Biggs H.C. (1971):** Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* 31, 87-96.
- Duncan J.R., Praise K.W., Mahaffey E.A. (1994):** Veterinary Laboratory Medicine (Clinical Pathology) 3rd ed. Iowa State University Press, U.S.A.
- Ganong W.F. (2005):** Review of Medical Physiology 22nd edition. McGraw – Hill Companies, Inc.
- Guyton A.C., Hall J.E. (2006):** Textbook of Medical Physiology 11th edition Elsevier Inc.
- Jain N.C. (1986):** Schalm's Veterinary Haematology 4th ed. Lea and Fabiger, Philadelphia.
- Johnson I. (1997):** The useful plants of West Africa, vol. 4 The Whitefriars Press Limited, Tonbridge, Kent TN9 1QR, Great Britain.
- Li Y., Xia I., Kuter D.J. (1999):** Interaction of thrombopoietin with the platelet complements receptor in plasma: binding, internalization, stability and pharmacokinetics. *Brit J. Haematol* 106: 345.
- Miladi – Gorgi H., Vafaei A.A., Rashidy – Pour A., Taherian A.A., Jarrahi M., Emami-Abargoei M. (2004):** Investigation of anxiolytic effects of aqueous extract of *Portulaca oleracea* in mice. *Iranian Journal of pharmaceutical research: Supplement* 2:57-57.
- Mitruka B.M., Rawnsley H. (1977):** Clinical, biochemical and haematological references values in normal experimental animals. Masson Publishing USA Inc. Pp. 53-54.

- Oyedeji K.O., Oluwole F.A. and Ademola S. (2007):** Effects of aqueous and methanolic extracts of *Portulaca oleracea* on intestinal smooth muscle. *Science Focus* vol 12 (1) Pp. 14 – 18.
- Polenakovic M., Sikole A. (1996):** Is erythropoietin a survival factor for red blood cells? *J. Am. Soc Nephrol* 7(8): 1178-1182.
- Radhakrishnan R., Zakaria M.N.M., Islam M.W. et al., (2001):** Neuropharmacological actions of *Portulaca oleracea*. *J Ethnopharmacol* 76:171-176.
- Reinhold J.G. (1953):** Manual determination of serum total protein, albumin and globulin fractions by the Biuret method *Standard Methods of Clinical Chemistry* (Academic Press, New York).
- Tonnesen H., Hejberg L., Frobenius S., Andersen J. (1986):** “Erythrocyte mean cell volume... correlation to drinking pattern in heavy alcoholics”. *Acta med Scand* 219(5): 515-8.
- Wong W. (1976):** Some folk-medicinal plants from Trinidad, *Econ. Bot* 30:103-142.
- Young N.S., Maciejewski J. (1997):** The path physiology of acquired aplastic anemia. *New Eng. J. Med.*, 336:1365.