



Lipid Profile and Hematological Assessment of Ethanol Extract of Whole *Merremicae hederacea* in Rats

Sulaiman, R.S.

¹Department of Biochemistry, IBB University, Lapai, Niger State, Nigeria

ABSTRACT

Immunological competence plays an important role in the progression of illness and treatment of diseases. The pathology of many diseases is associated with inflammatory responses. HDL-cholesterol is a potent anti-inflammatory agent known to reverse cholesterol transport from the arteries back to the liver for detoxification. The aim of this research was to evaluate the hypolipidaemic and immunomodulatory potentials of *Merremicae hederacea*. Crude Ethanol extract of *Merremicae hederacea* whole plant was administered to Wister rats daily over 4 weeks while the control group received normal saline. 3 rats were randomly selected from each group and sacrificed. Blood samples were collected for lipid profile and haematological analyses respectively. Cholesterol, triacylglycerol, HDL and LDL cholesterol were determined. Blood indices such as haemoglobin, haematocrit, red blood cell distribution width, red blood cell, white blood cell, lymphocyte, monocyte and granulocyte counts were determined. *M. hederacea* extract was able to significantly ($p > 0.05$) increase HDL-cholesterol concentration of the test compared to the control and also decreased significantly ($p > 0.05$) LDL-cholesterol concentration compared to the control. There was no significant ($p < 0.05$) difference in both white blood cell and red blood indices of the test compared to the control, however, there was a significant ($p > 0.05$) difference in the G/AG ratio of the test group compared to the control, thereby increasing chances of immune stimulation. *M. hederacea* extract has ability to increase serum HDL cholesterol levels and also stimulate the immune system. This outcome could not only potentially reduce the morbidity and mortality associated with cardiovascular diseases but also provide incentive for the development of a range of therapeutic agents designed specifically to increase HDL levels.

Keywords: *Merremicae hederacea*, cholesterol, haemoglobin, inflammation, high density lipoproteins,

INTRODUCTION

Derangement in normal serum lipid profile has been the cause of many illness and life threatening diseases. Diabetes mellitus, hypothyroidism, nephrotic syndrome, hypercholesterolemia, renal disease, obstructive liver disease, dysproteinemias and atherosclerosis are examples of such diseases and disorders that could result in abnormal serum lipid (Shabana *et al.*, 2020). Inflammation within the atherosclerotic plaque plays a major role in plaque rupture and the occurrence of acute coronary events (Bulbul *et al.*, 2018). Some medications, such as estrogens, progestins, androgens, retinoids, corticosteroids, beta-blockers, cyclosporin A, phenothiazines, anticonvulsants, diuretics, and certain antiviral agents are also known to modulate serum lipids (Osuji *et al.*, 2012 and Ames, 2012).

The primary role of immune system is to protect against infection/foreign invasion. Other roles

including tissue repair, wound healing, elimination of dead and cancer cells, and formation of the healthy gut microbiota are important contributions of the immune system (Simon *et al.*, 2015). Lymphocytes proliferate and differentiate at exponential rate, to maximize fighting power against antigen, resulting in the death of the majority of antigen-specific lymphocytes involved in the immune response. The few lymphocytes that survive become long-lived memory cells, which ensure that a second encounter with the same antigen is dealt with swiftly and effectively, due to the advantages they have over their naive predecessors in terms of their greater number, extended lifespan, more rapid response rate, superior proliferation capacity, and wider access to tissues (Yatim and Lakkis, 2015).

Received 24 March, 2021

Accepted 21 May, 2021

Address Correspondence to:

rukkysule82@gmail.com; +2348065863683

Immunological competence plays an important role in the progression of illness and treatment of diseases (Liu and Wei, 2010). The pathology of many diseases is associated with inflammatory responses. Inflammation is a defensive process initiated by the immune system against harmful stimuli (such as pathogen, damaged cells and toxic compounds) and acts by removing injurious stimuli and initiating repair or causing pathogenesis if unchecked (Hipp *et al.*, 2017). Although inflammatory response depends on the nature and location of the stimulus, the mechanism remains the same, which includes cell surface pattern receptor recognition, activation of inflammatory pathways, released and recruitment of inflammatory markers (Chen *et al.*, 2017). HDL-cholesterol is a potent anti-inflammatory agent known to reverse cholesterol transport from the peripheral vessels back to the liver for detoxification (Kosmas *et al.*, 2019). HDL produces anti-inflammatory effects in lesion macrophages suggesting a beneficial therapeutic effect of HDL in vivo (Fotakis *et al.*, 2019).

Green flora has remained the source of bioactive compound for the treatment and management of diseases in Africa since time immemorial. This has caught the global attraction towards natural product drug discovery for newer, safer and affordable medicine (Swargiary, 2017). Although in most cases no scientific studies have been done to prove the efficacy of these medicinal plants. *Merremicae hederacea* (Burm.f) Hallier is an herbaceous, glabrous twiner and climber, belonging to the family of convululaceae (Akobundu *et al.*, 2016). It is a medicinal plant common to riverine forest and grassland, often grown on sandy soil and it is distributed in Africa, Tropical Asia and Australia (Simoes *et al.*, 2015). The leaves are simple, ovate, 1.5-5cm long and 1.5-4cm wide (Charles *et al.*, 2012). The fruits are globoid or broadly conical capsules, indistinctive 4-angled, valves (Akobundu *et al.*, 2016). The seeds are pubescent or glabrous and 2.5mm long (Charles *et al.*, 2012). It is also used as a detergent by dwellers of riverine area in Africa before the advent of soap and the beautiful flowers are cultivated as ornamental plants. *Merremicae hederacea* is used locally for the treatment of fever. Grounded leaves mixed with turmeric are used to cure wounds and as an anti-inflammatory agent (Simoes *et al.*, 2015).

Despite the anti-inflammatory properties of this plant, scientific reports on its medicinal properties are few. The aim of this research was to evaluate the hypolipidaemic and immunomodulatory potentials of this plant.

METHODOLOGY

Collection and Extraction of *Merremicae hederacea* Plant

Merremicae hederacea was collected from Wuya in Lavun local government area of Niger State. Taxonomic identification and authentication was performed at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu- Abuja, where voucher specimens (NIPRD/H/6914) was deposited. The whole plant was air dried at room temperature and pulverized. 500 g of the pulverized sample was extracted with ethanol under reflux for 2 hrs. The filtrate was concentrated on a water bath and the solvent was completely evaporated in a fume cupboard to obtain the crude extract and then stored under refrigeration until required.

Animals Used

Healthy Wister rats in the range of 120-140g of either sex were obtained from Ahmadu Bello University Zaria, Nigeria. Ethical approval was obtained from Committee on Ethics for Medical and Scientific Research of Federal University of Technology, Minna, where the research was conducted. The principles, governing the use of laboratory animals as laid down by the Committee and also internationally accepted principles for use and care of laboratory animal as contained in the Canadian Council on Animal Care Guidelines and Protocol Review and international standard (1985) were adhered to. Twenty-four Wister rats were grouped into two groups of 12 rats each. The test group was given 200mg/kg of *Merremicae hederacea* crude extract while the control group received 0.1 mL of physiological saline daily for 4 weeks. The animals had access to pelleted feed and water *ad libitum*. On weekly basis, 3 rats were randomly selected from each group and sacrificed after overnight fast. Blood samples were collected in plain and heparinised bottle for estimation of lipid profile and haematological parameters respectively.

Lipid profile and Hematological assessment of *Merremicae hederacea* extract in rats

Moisture content of samples was determined by Randox diagnostic kits and a double beam UV visible spectrophotometer (Ebra Chem5v3 model) were used for the estimation of cholesterol, triacylglyceride (TAG), and high density lipoprotein (HDL), according to the methods of Thomas *et al.* (1998), Tietz (1990)

and Jacobs *et al.*, (1990) respectively, while low density lipoprotein(LDL) was obtained using the formula:

$$\text{LDL cholesterol (mmol/L)} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{2} - \text{HDL cholesterol}$$

Statistical Analysis

Results were analyzed using one-way ANOVA, with Statistical package MINITAB version 14. The results are expressed as mean \pm Standard error of mean ($\bar{x} \pm \text{SEM}$) of triplicates. Values with the same superscript are significantly ($p < 0.05$) same while those with different superscript are significantly ($p > 0.05$) different along the column.

RESULTS

Percentage yield of the crude extract of *M. hederacea* was 2.57 %.

Lipid profile of rat administered ethanol extract of *M. hederacea* for 28 days compared to the control is presented in Table 1. There was no significant ($p < 0.05$) difference in the total cholesterol and triacylglycerol concentration over the four weeks period. However, HDL concentration of test group were significantly (p

> 0.05) higher compared to control throughout the experimental period while LDL concentrations of the test group decreased significantly ($p > 0.05$) compared to the control.

Table 2 represent the white blood cell components of rats administered *M. hederacea* extract compared to the control over the period of four weeks. There was no significant difference in total leucocyte, lymphocyte, monocyte and granulocyte counts of the test group compared to the control. However, the granulocyte agranulocyte ratio was significantly higher compared to the control group.

The red blood cell indices of rats administered *M. hederacea* extract compared to the control is represented in Table 3. There was no significant difference in RBC count, hemoglobin count, hematocrit and red blood cell distribution width of the test group compared to the control.

Table 1: Lipid profile of rat administered *M. hederacea* for 28 days compared to the control

WEEK	Group	Cholesterol (mmol/L)	Triacylglycerol (mmo/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol (mmol/L)
Week1	<i>M. hederacea</i>	3.37 \pm 0.13 ^a	1.81 \pm 0.12 ^a	1.70 \pm 0.04 ^b	0.84 \pm 0.03 ^a
	Control	3.58 \pm 0.20 ^a	1.45 \pm 0.13 ^a	1.11 \pm 0.03 ^a	1.78 \pm 0.14 ^b
Week2	<i>M. hederacea</i>	3.46 \pm 0.05 ^a	1.04 \pm 0.01 ^a	1.98 \pm 0.02 ^b	1.01 \pm 0.02 ^a
	Control	3.64 \pm 0.12 ^a	1.55 \pm 0.20 ^a	1.20 \pm 0.07 ^a	1.73 \pm 0.05 ^b
Week3	<i>M. hederacea</i>	3.49 \pm 0.03 ^a	1.60 \pm 0.10 ^a	1.53 \pm 0.03 ^b	1.23 \pm 0.03 ^a
	Control	3.29 \pm 0.16 ^a	0.77 \pm 0.23 ^a	1.17 \pm 0.02 ^a	1.77 \pm 0.03 ^b
Week4	<i>M. hederacea</i>	2.83 \pm 0.10 ^a	1.21 \pm 0.26 ^a	1.68 \pm 0.18 ^b	0.67 \pm 0.02 ^a
	Control	2.73 \pm 0.02 ^a	1.42 \pm 0.16 ^a	1.11 \pm 0.01 ^a	0.97 \pm 0.04 ^b

Data are means \pm SE of three determinations. Values with the same superscript are significantly the same while those with different superscript are significantly different along the row.

Key: *M. hederacea* = test group, HDL =high density lipoproteins, LDL= low density lipoproteins

Table 2: White Blood Cell Indices of rats administered *M. hederacea* extract

WEEK	GROUP	WBC($\times 10^9/L$)	%LYM	%MONO	%GRA	GRA: AGR A
Week1	<i>M. hederacea</i>	18.14 \pm 1.83 ^a	78.73 \pm 5.41 ^a	6.23 \pm 1.64 ^a	15.03 \pm 3.92 ^a	0.177
	Control	12.66 \pm 4.12 ^a	79.96 \pm 4.50 ^a	10.93 \pm 2.83 ^a	9.13 \pm 2.34 ^a	0.100
Week2	<i>M. hederacea</i>	18.30 \pm 1.03 ^a	64.50 \pm 1.04 ^a	12.56 \pm 0.43 ^a	22.90 \pm 1.25 ^a	0.297
	Control	15.49 \pm 4.97 ^a	72.50 \pm 4.10 ^a	7.93 \pm 1.01 ^a	19.56 \pm 3.64 ^a	0.242
Week3	<i>M. hederacea</i>	16.84 \pm 1.23 ^a	58.10 \pm 7.66 ^a	16.26 \pm 0.89 ^a	25.63 \pm 6.83 ^a	0.345
	Control	12.72 \pm 1.74 ^a	72.96 \pm 2.69 ^a	11.83 \pm 2.51 ^a	15.20 \pm 0.47 ^a	0.179
Week4	<i>M. hederacea</i>	12.62 \pm 0.70 ^a	74.43 \pm 9.09 ^a	10.96 \pm 4.05 ^a	14.60 \pm 5.05 ^a	0.171
	Control	12.70 \pm 1.27 ^a	85.90 \pm 2.04 ^a	7.63 \pm 0.63 ^a	6.46 \pm 1.43 ^a	0.069

Data are means \pm SE of three determinations. Values with the same superscript are significantly ($p < 0.05$) the same while those with different superscript are significantly different along the row.

Key: *M. hederacea* = test group, WBC= white blood cells, %LYM= percentage lymphocytes, %MONO = percentage monocytes, %GRA= percentage granulocytes, GRA: AGR A = granulocytes: agranulocytes ratio.

Table 3: Red Blood Cell Indices of rats administered *M. hederacea*

Weeks	GROUP	RBC($\times 10^{12}/L$)	HGB (g/dl)	HCT (%)	RBWC (fL)
Week1	<i>M. hederacea</i>	6.77 \pm 0.55 ^a	12.46 \pm 1.20 ^a	39.41 \pm 4.17 ^a	18.80 \pm 0.40 ^a
	Control	6.65 \pm 1.92 ^a	10.83 \pm 3.21 ^a	39.40 \pm 1.34 ^a	21.66 \pm 2.42 ^a
Week2	<i>M. hederacea</i>	8.07 \pm 0.44 ^a	14.16 \pm 0.46 ^a	43.53 \pm 1.53 ^a	19.00 \pm 1.15 ^a
	Control	9.11 \pm 0.49 ^a	15.83 \pm 0.95 ^a	49.94 \pm 3.81 ^a	18.96 \pm 0.89 ^a
Week3	<i>M. hederacea</i>	7.00 \pm 1.09 ^a	9.71 \pm 4.10 ^a	43.70 \pm 1.38 ^a	23.53 \pm 1.27 ^a
	Control	6.66 \pm 0.64 ^a	12.43 \pm 1.37 ^a	42.48 \pm 3.31 ^a	24.06 \pm 0.89 ^a
Week4	<i>M. hederacea</i>	6.61 \pm 0.64 ^a	12.76 \pm 0.34 ^a	42.22 \pm 1.39 ^a	20.10 \pm 0.85 ^a
	Control	8.58 \pm 0.46 ^a	14.23 \pm 0.52 ^a	47.18 \pm 1.92 ^a	21.10 \pm 0.30 ^a

Data are means \pm SE of three determinations. Values with the same superscript are significantly ($p < 0.05$) the same while those with different superscript are significantly ($p < 0.05$) different along the row.

Key: *M. hederacea* = test group, RBC= red blood cells, HGB= hemoglobin, HCT = Hematocrit, RBWC= red blood cell distribution width.

DISCUSSION

The significant increase in HDL cholesterol level and a corresponding decrease in LDL cholesterol of the test group compared to the control throughout the study period could be due to the effect of the extract. Medication and nutrition have been reported to influence HDL composition (Bililioni *et al.*, 2016). Studies have shown that dietary cholesterol increased serum total and LDL cholesterol but not triacylglyceride concentration (Berger *et al.*, 2015). Dietary cholesterol is the culprit implicated in the increased blood cholesterol levels leading to elevated risk of cardiovascular diseases (Soliman, 2018). LDL cholesterol and high blood pressure are risk factors for

development of coronary heart disease (Ueda *et al.*, 2018). This study shows the absence of dyslipidaemia. Dyslipidaemia is characterized with total cholesterol ≥ 5.2 , LDL cholesterol ≥ 3.8 , HDL cholesterol < 0.9 and TAG ≥ 1.58 (mmol/L (Antwi-Baffour *et al.*, 2018). The HDL increasing property implies that *M. hederacea* has the ability to reverse cholesterol transport, decrease the risk of fatty acid deposit in the blood vessels, reduce the chances of atherosclerosis development and damage to blood vessels as well as improve metabolic health. The anti-inflammatory, cytoprotective and wound healing effects of HDL cholesterol which makes it a part of the innate host defense

system (Adams *et al.*, 2013 and Marz *et al.*, 2017) also implies that *M. hederacea* extract could enhance rapid healing of wound.

Although HDL has both anti-inflammatory and proinflammatory effects, the anti-inflammatory effect predominates over proinflammatory potential during inflammation. The anti-inflammatory effects of HDL-cholesterol are associated with reduced TLR (Toll-like receptor) 4 levels and reduced interferon receptor signaling while the proinflammatory effects occurs under conditions of extreme cholesterol depletion through a modified endoplasmic reticulum stress response that involves IRE1a (inositol-requiring enzyme 1a)/ASK1 (apoptosis signal-regulating kinase 1)/p38 MAPK (p38 mitogen-activated protein kinase) signaling (Fotakis *et al.*, 2019). The anti-atherosclerotic properties of HDL are likely the results of multifunction fulfilled by HDL including antioxidant; anti-inflammatory, anti-thrombotic and vaso regulatory actions in addition to its established ability to promote reverse cholesterol transport (Berthet *et al.*, 2014). Inflammation is a common pathogenesis of many diseases including malaria, typhoid, diabetes, arthritis, cancer, cardiovascular and bowel diseases (Chen *et al.*, 2017). Therefore, substance with anti-inflammatory property will have overwhelming effect on these diseases. Strategies that increased HDL levels could potentially decrease the incidence of cardiovascular disease (Rye, 2013). This upshot could not only potentially reduce the morbidity and mortality associated with cardiovascular and inflammatory diseases but also provide incentive for the development of a range of therapeutic agents designed specifically to increase HDL levels.

M. hederacea extract has ability to maintain a steady immune system by maintaining total leucocyte (white blood cell), lymphocyte, monocyte and granulocyte counts of the test group compared to the control group, while increasing immune system response potential by increasing in granulocytes agranulocytes ratio of the test compared to the control group, thereby enhancing the immune response potential in case of system against foreign invader. It will be expected that this extract should have an immune stimulating potential due to its high saponin concentration (Sulaiman, 2021). Saponins are used in immune system stimulation in patients with low immunity (Nurain *et al.*,

2017). WBC counts can be regarded as a marker of an increased synthesis of cytokines, acute phase reactants and the activation of the inflammatory signalling network involved in the pathogenesis of diseases (Babio *et al.*, 2013). Increase in total leukocytes, lymphocytes and neutrophils have a positive association with hypertriglyceridemia and low HDL levels (Shankar, *et al.*, 2004). However, in this study the extract was able to maintain total leucocyte count and also increase HDL cholesterol of the test group compared to the control.

Blood components such as white blood cells (WBC), granulocytes: agranulocyte ratio (G:AG), RBC and HGB are very significant, as they are essentially important parameters of the body's defense system. Although, the increase in granulocyte count was not significant in this study, people with low level of granulocytes are prone to infection (Deng *et al.*, 2016). Any substance capable of increasing these blood parameters can stimulate immune system (Chatterjee, *et al.*, 2013). Activated neutrophil can release reactive oxygen species (ROS) which attack invading microorganisms and the activated neutrophil undergo degranulation (Boly *et al.*, 2015), a cellular process that releases antimicrobial cytotoxic molecules from secretory vesicles (granules) found inside some cells. It is used by several immune cells, including granulocytes (neutrophils, basophils, and eosinophils) and mast cells (Sheshalam *et al.*, 2014).

The immune stabilizing potential is also evident by the lack of significant difference in total number of red blood cell (RBC), mean haemoglobin concentration (HGB), hematocrit and red blood cell distribution width (RBWC). Red blood cell possesses a unique role as gas carrier between blood and tissue. This function is altered in pathologic condition such as malaria, sickle cell anemia, diabetics, myocardial infarction and paroxysmal nocturnal hemoglobin (Tomaiuolo, 2014). Hemoglobin concentration and hematocrit are two parameters used to assess oxygen carrying capacity of the blood, to either diagnose anemia or polycythemia. Oxygen transport is the most important function of hemoglobin in the red blood cells and oxygen affinity has a positive correlation with hemoglobin concentration (Di Caprio *et al.*, 2015). Since administration of *M. hederacea* extract had no significant effect on both hemoglobin concentration and hematocrit

of the test group compared to the control, administration of *M. hederacea* extract will not hinder oxygen transport. Similarly, red blood cell characteristics are used to monitor and diagnose a wide range of conditions including malnutrition, inflammation and cancer (Malka *et al.*, 2014). The RBWC is one of such characteristics that was unaffected by administration of *M. hederacea* extract in this study. The observed stability in RBC count is an evidence to show *M. hederacea* extract will not have a negative impact on RBC count when explore for its therapeutic purposes in the treatment of illness that causes anemia, of which malaria, diabetics are inclusive.

Conclusion

M. hederacea extract has the ability to increase serum HDL cholesterol levels and also stimulate the immune system. This outcome could not only potentially reduce the morbidity and mortality associated with cardiovascular diseases but also provide incentive for the development of a range of therapeutic agents designed specifically to increase HDL levels and boost the animal immune responses.

REFERENCES

- Adenekan, M. K., Oguntoyinbo, S. I., Adams, V., Besler, C., Fischer, T., Riwanto, M., Noack, F., Hollriegel, R., Oberbach, A., Jehmlich, N., Volker, U., Winzer, E. B., Lenk, K., Hambrecht, R., Schuler, G., Linke, A., Landmesser, U., Erbs, S., (2013). Exercise training in patients with chronic heart failure promotes restoration of high-density lipoprotein functional properties. *Circulation Research*, 113(12):1345–1355 doi: 10.1161/CIRCRESAHA.113.301684.
- Akobundu, O. I., Ekeieme, F., Agyakwa, C. W., Ogazie, C. A., (2016). A handbook of West African Weeds, *International Institute of Tropical Agriculture*, Ibadan, pp133.
- Ames, R. (2012). Effect of diuretic drug on lipid profile. *Drugs*, 36, 33-40.
- Antwi-Baffour, S., Kyeremeh, R., Boateng, S. O., Annison, L., and Seidu, A. M., 2018. Haematological parameters and lipid profile abnormalities among patients with Type-2 diabetes mellitus in Ghana. *Lipids in Health and Disease*, 17:283. doi:10.1186/s12944-018-0926-y.
- Babio, N., Ibarrola Jurado, N., Bullo, M., Martinez-Gonzalez, M. A., Warnberg, J., Salverria, I., Ortega-Calvo, M., Estruch, R., Serra-Majem, L., Casas, M. I., Sorli, J. V., Salas-salvado, J., (2013). White blood cell counts as risk markers of developing metabolic syndrome and its components in the predimed study, *PLOS ONE*, 8(3):e58354. Doi:10.1371/journal.pone.0058354.
- Berger, S., Raman, G., Vishwanathan, R., Jacques, P. F., Johnson, E. J., (2015). Dietary cholesterol and cardiovascular disease: a systemic review and meta-analysis. *American journal of Clinical Nutrition*, 102:276-294. Doi:10.3945/ajcn.114.100305.
- Berthet, S., Spahis, S., Levy, E., (2014). High Density Lipoproteins Function: Lessons from proteomics Approach, *Journal of Glycomics and Lipidomics*, 4(3), 1-8. Doi:10.4172/2153-0637.1000118.
- Bibiloni, M., Salas, R., Garza, Y. E, Villareal, J. Z., Sureda, A., Tur, J. A., (2016). Serum lipid profile, prevalence of Dyslipidemia and associated risk factors among Northern Mexican Adolescents, *Nutrition*, 63(5), 544-549.
- Boly, R., Franck, T., Kohnen, S., Lomgo, M., Guasson, I. Dubois, J., Sertejn Didier, S., and Mouths-Mickalad, A., (2015). Evaluation of Antracanthin and anti-inflammatory activities of ethylacetate and butanolic extractions of *Agelanthus dodoneifolius* (DC.) Polhill and Wiens (Loranthaceae) using equine myeloperoxidase and both PMA-activated neutrophils and HL-60 cells. *Evidence Based complementary and Alternative medicines*, 1-9. doi.org/10.1155/2015/7075 24.
- Bulbul, M. C., Dage, T., Afsar, B., Ulu, N. N., Kuwabara, M., Covic, A., Kanbay, M., (2018). Disorders of Lipid Metabolism in Chronic Kidney Disease. *Blood Purification* 2018;46:144–152. Doi:10.1159/000488816.

- Charles, A. Joseph, M., Alex, R. V., (2012). Phytochemical investigation of *Merremia hederacea*, *Journal of Natural Product and Plant Resource*, 2, 486-489.
- Chatterjee, S. Chatterjee, S., Dey, K. K., and Duttas, S., (2013). Study of antioxidant activity and immune stimulating potency of ethnomedical plants *Cassia alata* (L) Robx, *Medicinal and Aromatic Plants*, 2(4)1-6 doi:104172/2167-04121000131.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z. Jeng, J., Li, Y., Wang, X., Zhao, L., (2017). Inflammatory responses and inflammation-associated diseases in organs, *Oncotarget*, 9(6)7204-7218.
- Deng, Y., Ye, J., Luo, Q., Huang, Z., Peng, Y., XIONG, G., Guo, Y., Jiang, H., Li, J., (2016). Low density granulocytes are elevated in mycobacterial infection and associated with the severity of tuberculosis. *PLOS ONE*, e0153567. Doi:10.1371/journal.pone.0153567.
- Di Caprio, G., Stokes, C., Higgins, J. M., Schonbrun, E., (2015). Single-cell measurement of red blood cell oxygen affinity. *Medical Sciences*, 112(32):9984-9989. Doi:10.1073/pnas.1509252112/./Desupplemental.
- Fotakis, P., Kothari, V., Thomas, D. G., Westerterp, M., Molusky, M. M., Altin, E., Abramowicz, S., Wang, N., He, Y., Heinecke, J. W., Bornfeldt, K. E., Alan R. Tall, A. R., (2019). Anti-inflammatory effects of HDL (High-Density Lipoprotein) in macrophages predominate over proinflammatory effects in atherosclerotic plaques. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 39,e253–e272. doi:10.1161/ATVBAHA.119.313253.
- Hipp, S., Tai, Y. T., Blanset, D., (2017). A current view on inflammation, *Nature Immunology*, 18(8)825. Doi:/10.1038/ni3798.
- Jacobs, D. S., Kasten, B. L., De Moh, W. R., Wolfson, W. L., (1990). *Laboratory and test Handbook*, Lexi company Inc: Hudson (Cleveland), pp219.
- Kosmas, C. E., Martinez, I., Sourlas, A., Bouza, K.V., Campos, F. N., Torres, V., Montan, P. D., Guzman, E., (2018). High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs in Context*, 2018; 7: 212525. DOI: 10.7573/dic.212525.
- Liu, A., Wei, L., (2010). Leukocyte infiltration as a surrogate marker for diagnosis of invasion, *International Journal of Biological Sciences*, 6(3)225-227.
- Malka, R., Delgado, F. F., Manalis, S. R., Higgins, J. M., (2014). *In vivo* volume and hemoglobin dynamics of human red blood cells. *PLoS Computational Biology*, 10(10):e1003839. doi:10.1371/journal.pcbi.1003839.
- Marz, W., Weber, M.E., Sharnagl, H., Spcer, T., Zewinger, S., Ritsch, A., Parhofer, K.G., Eckardstein, A., Landmesser, I., Laufs, U., (2017). HDL cholesterol: reappraisal of clinical relevance. *Clinical Research in Cardiology*. 106:663-675. Doi: 10.1007/s0039-017-1106-1.
- Nurain, I.O., Bewaji, C. O., Johnson, J. S., Davenport, R. D., (2017). Potentials of three ethnomedicinal plants as antisickling agents. *Molecular Pharmaceutics*, 14,172-182.
- Osuji, C. U., Omejua, E. G., Onwubuya, E. I., Ahaneku, G. I., (2012). Serum Lipid Profile of Newly Diagnosed Hypertensive Patients in Nnewi, South-East Nigeria. *International Journal of Hypertension*, 2012, 710486, 7 pages.doi:10.1155/2012/710486.
- Rye, K., (2013). High density lipoproteins, structure, function and metabolism: a new thematic series. *Journal of Lipid Research*, 54.2031-2033. Doi:10.1194/jlrE041350.
- Shabana, S., Ullah, S., Sumbal S., 2020. The abnormal lipid profile in obesity and coronary heart disease (CHD) in Pakistani subjects. *Lipids in Health and Disease*, 19, 73. Doi:10.1186/s12944-020-01248-0.
- Shankar, A., Klein, B. E., Klein, R. 2004. Relationship between white blood cell count and hypertension. *American Journal of Hypertension*, 17,233-239. Doi:

- Sheshalam, A., Srivasta, N., Mitchell, T., Lacv, P., Eitzen, G., (2014). Granule protein processing and regulating secretion in neutrophils, *Frontier in Immunology*, 5(448):1-11.
Doi:10.3389/fimmu.2014.00448.
- Simoos, A. R., Culham, A., Carine, M., (2015). Resolving the unsolved tribes: a molecular phylogenetic framework for the Merremiaceae (convululaceae), *Botanical Journal of Linnean Society*, 179, 374-387.
- Simon, A. K, Hollander, G. A., Mcmichael, A., (2015). Evolution of the immune system in humans from infancy to old age, *Proceedings of Royal Society B* 282: 2014 3005 doi: Org/10.1098 /rspd.2014.3005.
- Soliman, G. A., (2018). Diatary cholesterol and lack of evidence in cardiovascular disease, *Nutrients*,10:780.
Doi:10.3390/nu10060780.
- Sulaiman, R. S. (In press) Safety evaluation of crude ethanol extract of *Merremia hederacea* in albino rats. *Journal of Natural and Applied Sciences*. X, XX.
- Swargiary, A. (2017). Recent trends in traditionally used medicinal plants and drug discovery, *Asian Journal of Pharmacy and Pharmacology*, 3;4.
- Thomas, L. (1998). *Clinical Laboratory Diagnostics*, 1st edition, TH-Books Verlagsgesellschaft mbH, Frankfurt/Main, Germany, pp169.
- Tietz, N. W.(1990). *Clinical guide to laboratory tests*, 2nd edition, W.B Saunders company, Philadepia,USA, pp554-556.
- Tomaiuolo, G. (2014). Biomechanical properties of red blood cells in health and disease towards microfluidics. *Biomicrofluidics*, 8(51150)1-19.
Doi:10.1063/1.4895755.
- Ueda, P., Gulayin, P., and Danaei, G. (2018). Long term moderately elevated LDL-cholesterol and blood pressure and risk of coronary heart disease, *PLOS-ONE*, 13(7):e0200017.
- Yatim, K. M., and Lakkis F. G. (2015). A brief journey through the immune system. *Clinical Journal of American Society of Nephrology*, doi: 10:2215/CJN.10031014.