International Journal of Biological Sciences and Applications
2018; 5(2): 29-33
http://www.aascit.org/journal/ijbsa
ISSN: 2375-3811

AASCIT American Association for Science and Technology

Inhibition of the Expressions of Splenic TNF-alpha Receptor Superfamily 8, CD3 and CD20 by Ethanolic Extract of *Xylopia aethiopica*

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Citation

Babatunde Joseph Oso, Oyedotun Moses Oyeleke, Adenike Temidayo Oladiji. Inhibition of the Expressions of Splenic TNF-alpha Receptor Superfamily 8, CD3 and CD20 by Ethanolic Extract of *Xylopia aethiopica*. *International Journal of Biological Sciences and Applications.* Vol. 5, No. 2, 2018, pp. 29-33.

Received: February 21, 2018; **Accepted**: March 26, 2018; **Published**: May 10, 2018

Abstract: This study was designed to investigate the effects of ethanolic extract of *Xylopia aethiopica* (Dunal) A. Rich on the expressions of splenic tumour necrosis factor-alpha receptor superfamily 8 (TNFRSM8), CD3 and CD20 in Wistar rats with induced acute inflammation by injection of turpentine oil (TURP). The splenic TNF-alpha receptor superfamily 8 (TNFRSM8), CD3 and CD20 were identified through qualitative immunohistochemical staining. The results revealed that the extract reduced the expressions of TNFRSM8, CD3 and CD20 in the spleen of Wistar rats. In summary, the results of the present study showed that prophylactic oral administration of the fruit of *X. aethiopica* (Dunal) A. Rich could have protective effects on splenic tissues exposed to TURP-induced acute inflammation.

Keywords: *X. aethiopica* (Dunal) A. Rich, TNF-Alpha, TNF-Alpha Receptor, Turpentine Oil, CD3 and CD20

1. Introduction

Cell survival, apoptosis, proliferation and differentiation have been established to be influenced by the activations of the tumour necrosis factor-alpha receptor (TNFR) members [1]. The expression of TNF-alpha receptor superfamily 8 (TNFRSM8) (otherwise known as CD30) commonly initiated by the activations of CD3 and CD20 has been associated with increased risks of non-Hodgkin lymphoma, perhaps through the activation of nuclear factor kappa beta (NF-kappaB) transcription factor by tumour necrosis factor-alpha (TNF-alpha), a pro-inflammatory cytokine that is critical in inflammatory and apoptotic responses in uncontrollably cell proliferation and cancer progression [2, 3]. Modulation of TNF-alpha secretion and the functions of their TNFRs can be innovative targets in therapeutic prevention, management and treatment of a variety of diseases associated with immune disorders. The use of herbs has been recommended to be an approach in the modulation of cytokine expressions [4].

Turpetine oil (TURP) is a natural derivative of resin from pine trees (*Pinus* spp.). It is largely composed of monoterpenes and pinene [5]. Earliest works by Berenblum (1935) and Fuchs (1966) had, respectively, reported that dermal application of TURP on mice resulted in thickening of skin and loss of hair and the intramuscular injection in rat resulted in immunotoxicity, a pathological disorder that was characterised by inflammation and damage of granulation structures of macrophages [6, 7]. Also, repetitive injections of TURP into rats lead to a rise in the dry weight of the adrenals, liver, spleen, and involution of the thymus; changes that were akin to those observed in the tumour-bearing rat [8]. Nevertheless, TURP has been used medicinally as an aroma-therapeutic agent in the treatment of symptoms related to common cold and in the prevention of bovine babesiosis [9, 10]. The reported therapeutic claims might be owing to its pro-inflammatory and immune-stimulatory potential at relatively lower dose. Prophylactic and therapeutic administrations of some botanical extractives have been reported to significantly alleviate oxidative stress and acute inflammation induced by single injection of TURP in experimental rats [11, 12]. Consequently, TURP-induced acute

inflammation has been used as an experimental model for variety of inflammatory diseases [12–14]. Several studies have revealed that the botanicals and phytochemicals such as cannabis, resveratrol, curcumin, catechins etc. modulate the secretions of multiple cytokines [12, 15-17].

The fruit of *X. aethiopica* (Dunal) A. Rich has been used in orthodox medicine in various regions of West Africa for the management of various diseases associated with dysfunctional inflammatory response [12]. Despite the wide acceptance of *X. aethiopica* (Dunal) A. Rich in orthodox medicine, its mode of actions with respect to the expressions of cytokine bio-signalling is considerably less understood. Thus, the aim of this study was to investigate the effect of oral administration of ethanolic extract of the dried fruit of *X. aethiopica* (Dunal) A. Rich on the inhibition of the expressions of splenic TNF-alpha receptor superfamily 8, CD3 and CD20 in Wistar rats with acute inflammation.

2. Methodology

2.1. Plant Materials

The fruits of *X. aethiopica* (Dunal) A. Rich (Annonaceae), commonly called Negro pepper or Ethiopia pepper and locally known in Hausa as *Kimbaa*, in Igbo as *Uda* and in Yoruba as *Erínje* or *Èèrù* were collected from different locations in the North Central Region of Nigeria. The samples were authenticated at the Department of Plant Biology, University of Ilorin with the voucher number UIH001/1089. Powdered sample of the fruit of *X. aethiopica* (Dunal) A. Rich was extracted in ethanol for seventy-two hours with intermittent shaken with the aid of a shaker. The mixture was filtered using filter paper to remove the solutes and extractant from the plant residue. The crude extract was dried under vacuum using rotary evaporator.

2.2. Animal Handling

Fifteen (15) Wistar rats were randomly divided into three different groups with five animals per group: A (control), B (induced + extract administration) and C (induced with no extract administration). Daily dose of 100mg/kg of the extract prepared in 5% dimethylsulphoxide (DMSO) solution was administered orally for fourteen days to the animals in group B. On the fifteenth day of the experiment, the rats were injected with 0.1 ml of normal saline (group A) or 0.1 ml of TURP, (groups B and C) to induce acute inflammation. Seventeen hours after the TURP injection, fasted animals in all the groups were sacrificed under diethylether anaesthesia. The spleens were excised, washed twice with 10% formaldehyde to remove residual blood. A slice of each spleen was taken and fixed in 10% formaldehyde and randomly processed for immunohistochemical examinations.

2.3. Immunohistochemical Analysis

The qualitative immunohistochemical analysis on the spleens was carried out at the Histopathology section of the University of Ilorin Teaching Hospital, Ilorin. The principle is based on the interaction of the monoclonal antibodies of TNFRSM8, CD20 and CD3 with the intracytoplasmic portion of the specific antigens expressed by the spleen.

3. Results

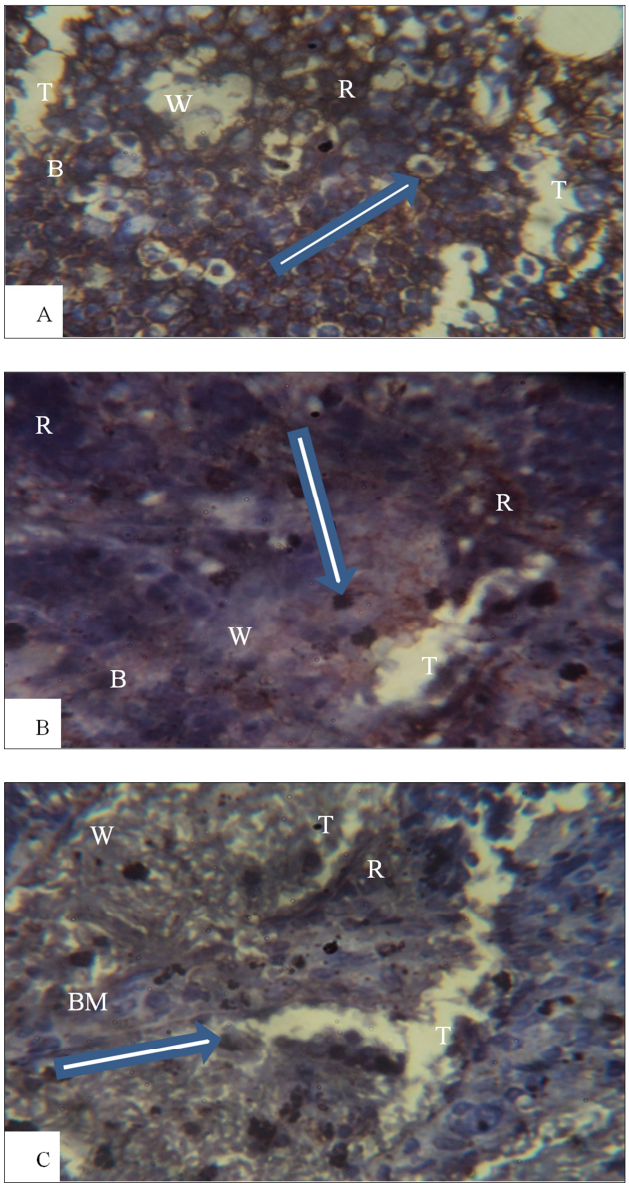

Figure 1. *Photomicrograph of splenic TNFRSM8 of Wistar rats administered ethanolic extract of X. aethiopica (Magnification×40).*

(A) Control (B) Ethanolic extract 100mg/kg body weight (C) Induced control showing higher expression of tumour necrosis factor receptor (TNFRSM8). Arrow indicates the TNFRSM8 immunopositivity in the basement membrane (BM), (RP) represents red pulp, (T) represents trabecula, and (WP) represents white pulp.

Immunohistochemistry staining of the spleen showed significantly higher immunopositivity of TNFRSM8 in the splenic basement membrane of the rats in the group that received the TURP injection without prior administration of the extract with significant architectural distortion in contrast to the animals in the control group. Lesser distortion was

identified in the architecture of the animals in the group with prior administration of the extract (Figure 1). The results presented in Figures 2 and 3 showed the suppressive effect of the pre-treatment of ethanolic extract of the fruit of *X. aethiopica* (Dunal) A. Rich on the activations of CD3 and CD30 in the spleen. Single injection of TURP significantly increased the expressions of CD 20 and CD 3 compared to the control. However, oral administration of the ethanolic extract at 100mg/kg body weight suppressed the expressions of CD3 and CD30 to levels comparable to those in the control.

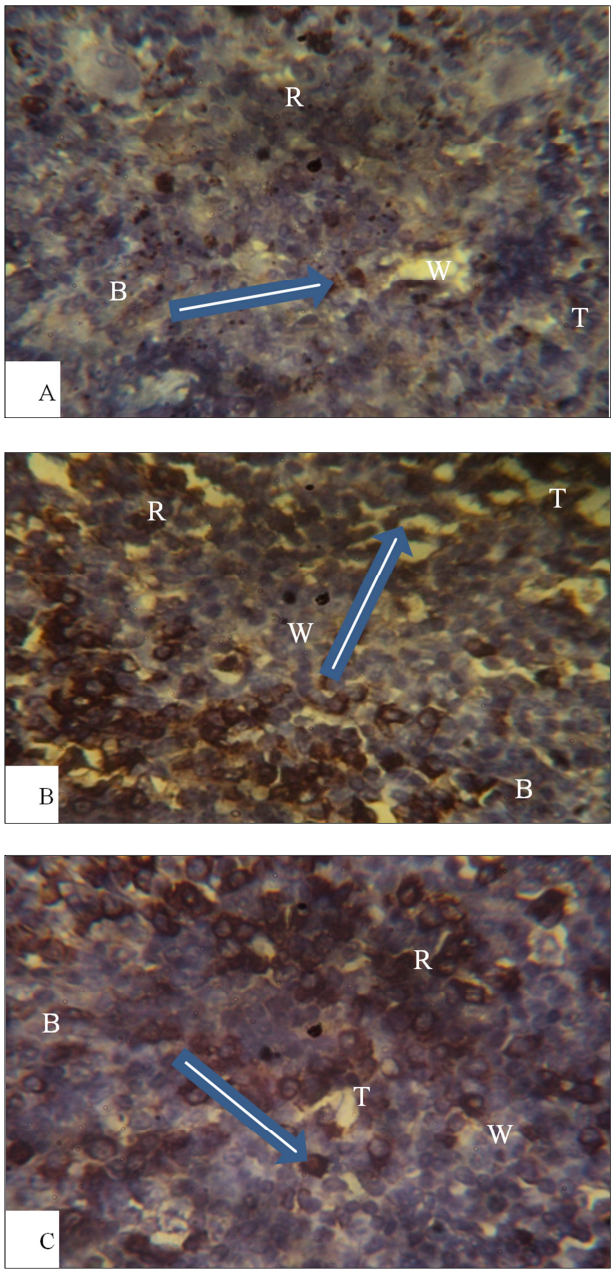

Figure 2. *Photomicrograph of splenic CD 3 of Wistar rats administered ethanolic extract of X. aethiopica (Magnification×40).*

(A) Control (B) Ethanolic extract 100mg/kg body weight (C) Induced control showing higher expression of T lymphocytes. Arrow indicates the CD3 immunopositivity in the basement membrane (BM), (RP) represents red pulp, (T) represents trabecula, and (WP) represents white pulp.

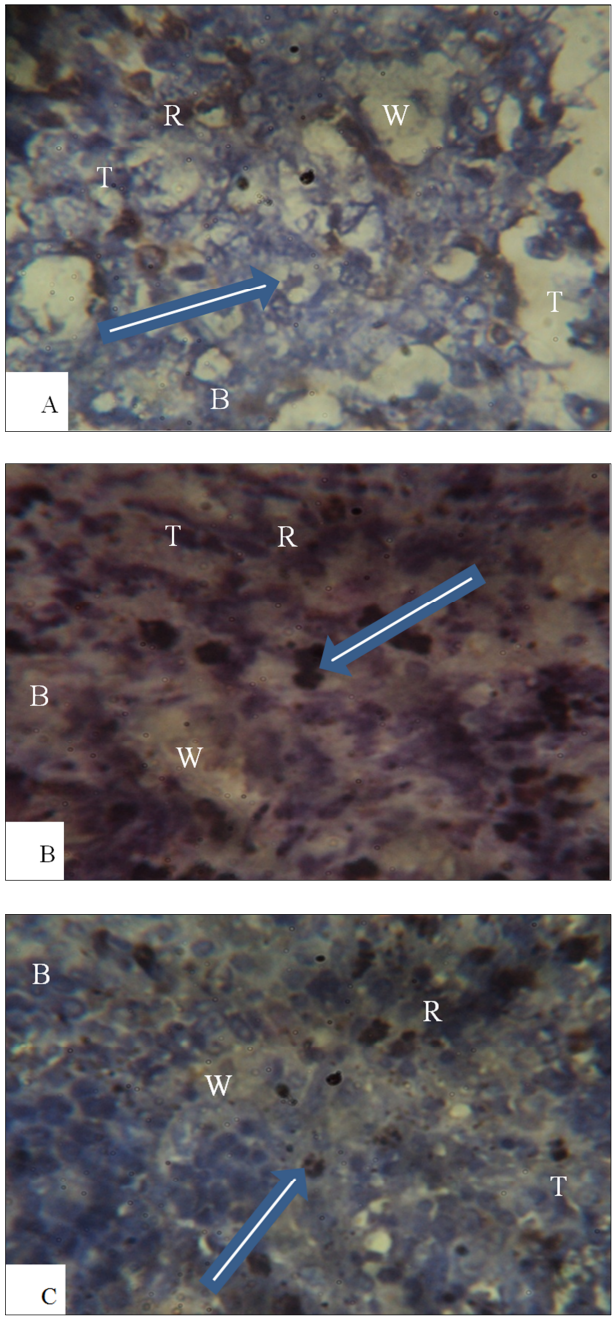

Figure 3. *Photomicrograph of splenic CD 20 of Wistar rats administered ethanolic extract of X. aethiopica (Magnification×40).*

(A) Control (B) Ethanolic extract 100mg/kg body weight (C) Induced control showing higher expression of B lymphocytes. Arrow indicates the CD20 immunopositivity in the basement membrane (BM), (RP) represents red pulp, (T) represents trabecula, and (WP) represents white pulp.

4. Discussion

The spleen plays vital roles in immune modulation. It is the largest lymphoid organ in the body. Induction of inflammatory response by TURP had been shown to affect the expressions of splenic proteins that are involved in iron homeostasis [18]. The most common causes of splenic abnormalities include release of inflammation-induced signaling molecules, infiltration with cancer cells, liver

diseases, splenic vein thrombosis and infections [19-21]. Splenic abnormalities include splenomegaly, splenic involution and asplenia. Irrepressibly expressions of T cells have been connected with the mechanisms responsible for splenomegaly [22]. Moreover, hyperactivation of splenic macrophages and secretions of pro-inflammatory and pro-fibrogenic factors such as IL-1β, IFN-γ, TNF-α and TGF-β1 had also been presented as potential facilitators of cirrhosis-associated hypersplenism [23, 24]. Various plant extractives have been used in the treatment of disorders associated with inflammatory response. The proposed mechanisms of action include polarisation of the T_H0 lymphocyte population towards the expression of T_H1 immune response and modulation of expressions of pro-inflammatory cytokines and anti-inflammatory cytokines [12, 25-27].

Acute inflammation occurs as an instant and rapid response to infections or injuries; the response may involve the activation of an immune response which must be terminated when it is no longer needed, as its hyper-activations may result in chronic inflammation, and cellular/tissue destruction [28, 29]. Appropriate functioning of anti-inflammatory mediators, pro-inflammatory receptor antagonists, cytokine receptors and apoptosis of pro-inflammatory cells are crucial in regulation of inflammation responses; elsewise, hyper-activations of immune response could lead to the evolution of inflammatory pathologies most especially autoimmune diseases. Various remedies such as steroids, non-steroidal anti-inflammatory drugs, neutralising monoclonal antibodies and a number of botanicals and phytocompounds have been exploited as anti-inflammatory agents to keep inflammation in check beyond the physiological requirement [30]. Ethnobotanically, the fruit of *X. aethiopica* (Dunal) A. Rich has been used medicinally and different claims had been attributed to its medicinal properties. The metabolomic profiling of the fruit extractives showed the presence of many anti-inflammatory phytocompounds such as ferentinide, betulin, hydrocortisone acetate β-pimaric acid, xylopic acid (kaur-16-ene), quercetin, chlorogenic acid, ellagic acid, caffeic acid, rutin, apigenin and kaempferol [31]. Earlier reports on the suggestive anti-inflammatory properties of *X. aethiopica* (Dunal) A. Rich associated its analgesic and antipyretic effects to the inhibition of synthesis and release action of pro-inflammatory molecules [32]. TNF-alpha, for instance, exerts its effects via TNF-alpha receptors (TNFRs) which are expressed by all nucleated cells [33]. Moreover, botanicals and phytocompounds such as saponins, terpenoids and phenolics have been reported to modulate the immune cell proliferation through their actions on cytokine receptors, activities of cytokines, and other mediators of immune response [1, 15-16]. In the present study, the ethanolic extract at 100mg/kg of body of rat was shown to decrease the expression of TNFRSM8 in the spleen of rats with acute inflammation, compared to the induced control. There was a notable decrease in the expressions of CD3 (T cells) and CD20 (B cells). The acute inflammation was induced by single injection of TURP, an essential oil from pines that has been demonstrated to initiate inflammatory response [5]. The decrease in the expression of the TNFRSM8 in groups A and B is believed to be linked to the lesser activations of T cells and B cells [34].

5. Conclusion

The results of the present study provide experimental validation that prophylactic administration of *X. aethiopica* (Dunal) A. Rich could protect against splenic damage that could be caused by exposure to pro-inflammatory inducers through modulation of the expressions of T cells and B cells. The study suggests that regular consumption of the fruit of *X. aethiopica* (Dunal) A. Rich could be encouraged in the management of diseases associated with immune disorders. Nonetheless, additional investigations are needed on safely use of the fruit as medication. Further study on the possible mechanism of action of the extract and the signaling pathway is similarly recommended.

Acknowledgements

The authors appreciate Mr. A. Fowotade of Histopathology section of the University of Ilorin Teaching Hospital, Ilorin for his professional assistance in the immunochemistry techniques.

References

[1] Hehlgans T and Klaus Pfeffer K (2005) The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 115 (1): 1–20.

[2] Wang SS, Purdue MP, Cerhan JR, Zheng T, Menashe I, Armstrong BK, Lan Q, Hartge P, Kricker A, Zhang Y, Morton LM, Vajdic CM, Holford TR, Severson RK, Grulich A, Leaderer BP, Davis S, Cozen W, Yeager M, Chanock SJ, Chatterjee N, and Rothman N (2009) Common gene variants in the tumour necrosis factor (TNF) and TNF receptor superfamilies and NF-kB transcription factors and non-Hodgkin lymphoma risk. PLoS One; 4 (4):e5360.

[3] Chiu BC and Weisenburger DD (2003) An update of the epidemiology of non-Hodgkin's lymphoma. Clin Lymphoma. 4: 161–168.

[4] Spelman K, Burns JJ, Nichols D, Winters N, Ottersberg S and Tenborg M (2006) Modulation of Cytokine Expression by Traditional Medicines: A Review of Herbal Immunomodulators Alternative Medicine Review 11 (2): 128-150.

[5] Prasad VG, Vivek C, Anand Kumar P, Ravi Kumar P, and Rao GS (2015) Turpentine oil induced inflammation decreases absorption and increases distribution of phenacetin without altering its elimination process in rats. Eur J Drug Metab Pharmacokinet. 40 (1): 23-8.

[6] Berenblum I (1935) Experimental inhibition of tumour induction by mustard gas and other compounds. J. Pathol. Bacteriol. 40: 549-558.

[7] Fuchs U (1966) Zur submikroskopischen struktur der makrophagen im terpentinölgranulom der ratte. Virchows Arch. Path. Anat. 341: 108-114.

[8] Kampschmidt RF, Goodwin WL and Mayne MA (1960) Iron Metabolism and Organ Weights of Rats With Turpentine Inflammation. Journal of the National Cancer Institute, 24 (3): 673–678.

[9] Xrefer. (2001) Vicks VapoRub (Procter & Gamble). Available on the Internet at: http://www.xrefer.com/entry/475982.

[10] Biggs LJ, Carrington CAP and Naidoo V (2014) Evaluation of medicinal turpentine used for the prevention of bovine babesiosis in southern KwaZulu-Natal and the eastern Free State. Onderstepoort Journal of Veterinary Research. 18 (1): Art. #705, 6 pp.

[11] Coneac A, Orasan MS, Leucuta Dc, Decea N, Filip M, Mihu Cm, Muresan A, Orasan Ri, and Moldovan M (2017) Effect of Curcumin on Oxidative Stress in a Model of Turpentine Induced Acute Experimental Inflammation. Not Bot Horti Agrobo. 45 (1): 65-74.

[12] Oso BJ, Oyewo EB and Oladiji AT (2017) Ethanolic, n-hexane and aqueous partitioned extracts of *Xylopia aethiopica* fruit modulated inflammatory responses in turpentine oil induced acute inflammation in male Wistar rats. International Journal of Research in Health Sciences. 5 (2): 1-10.

[13] Kalra R, Singh SP, Pena-Philippides JC, Langley RJ, Razani-Boroujerdi S, and Sopori ML (2004) Immunosuppressive and Anti-Inflammatory Effects of Nicotine Administered by Patch in an Animal Model. Clin Vaccine Immunol. 11 (3): 563-568.

[14] Nagaoka MR Kouyoumdjian M and Borges DR (2000) The hepatic clearance of recombinant tissue-type plasminogen activator decreases after an inflammatory stimulus Brazilian Journal of Medical and Biological Research. 33: 119-125.

[15] Raduner S, Majewska A, Chen JZ, Xie XQ, Hamon J, Faller B, Altmann KH, and Gertsch J (2006) Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. J. Biol. Chem. 281 (20): 14192–206.

[16] Hatcher H., Planalp R., Cho J., Torti FM and Torti SV (2008) Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci. 65 (11): 1631- 1652.

[17] Oyewo EB, Akanji MA and Adekunle AS (2012) Immunomodulation Capabilities of Aqueous Leaf Extract of Phyllanthus amarus in male Wistar Rats Report and Opinion; 4 (1): 22-37.

[18] Sukumaran A, Venkatraman A and Jacob M (2012) Inflammation-induced effects on iron-related proteins in splenic macrophages and the liver in mice. Blood Cells Mol Dis. 49 (1): 11-9.

[19] Bashour FN, Teran JC and Mullen KD (2000) Prevalence of peripheral blood cytopenias (hypersplenism) in patients with nonalcoholic chronic liver disease. Am J Gastroenterol. 95 (10): 2936–2939.

[20] Li L, Duan M, Chen W, Jiang A, Li X, Yang J and Li Z (2017) The spleen in liver cirrhosis: revisiting an old enemy with novel targets. J Transl Med. 15: 111.

[21] Chapman J and Azevedo AM (2018) Splenomegaly. [Updated 2017 Oct 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430907/.

[22] Nomura Y, Kage M, Ogata T, et al. (2014) Influence of splenectomy in patients with liver cirrhosis and hypersplenism. Hepatol Res. 44 (10):E100–E109.

[23] Jiang A, Zhang S, Li Z, et al. (2011) miR-615-3p promotes the phagocytic capacity of splenic macrophages by targeting ligand-dependent nuclear receptor corepressor in cirrhosis-related portal hypertension. Exp Biol Med. 236 (6): 672–680.

[24] Ren S, Zhang S, Li M, et al. (2013) NF-κB p65 and c-Rel subunits promote phagocytosis and cytokine secretion by splenic macrophages in cirrhotic patients with hypersplenism. Int J Biochem Cell Biol. 45 (2): 335–343.

[25] Dey P and Chaudhuri TK (2014) In vitro modulation of TH1 and TH2 cytokine expression by edible tuber of Dioscorea alata and study of correlation patterns of the cytokine expression. Food Science and Human Wellness. 3 (1): 1-8.

[26] Ahmad W, Jantan I, Kumolosasi E and Bukhari SNA (2015) Immunostimulatory effects of the standardized extract of Tinospora crispa on innate immune responses in Wistar Kyoto rats. Drug Des Devel Ther. 9: 2961–2973.

[27] Varma RS, Guruprasad KP, Satyamoorthy K, Kumar LMS, Babu UV, and Patki SP (2016) IM-133N modulates cytokine secretion by RAW264.7 and THP-1 cells. J Immunotoxicol. 13 (2): 217–225.

[28] Butcher EC and Picker L J (1996) Lymphocyte homing and homeostasis. Science 272, 60-66.

[29] Bafna AR and Mishra SH (2005) Immunomodulatory activity of methanolic extract of roots of Cissampelos pareira Linn. Ars. Pharm., 46, 253-262.

[30] Balkwill F and Mantovani A (2010) Cancer and inflammation: implications for pharmacology and therapeutics. Clin Pharmacol Ther 87, 401-406.

[31] Oso BJ, Boligon AA and Oladiji AT (2018) Metabolomic profiling of ethanolic extracts of the fruit of *Xylopia aethiopica* (Dunal) A. Rich using Gas Chromatography and High-Performance Liquid Chromatography techniques. Journal of Pharmacognosy and Phytochemistry. 7 (1): 2083-2090.

[32] Woode E, Ameyaw EO, Boakye-Gyasi E, and Wonder KM (2012) AbotsiAnalgesic effects of an ethanolic extract of the fruits of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) and the major constituent, xylopic acid in murine models J Pharm Bioallied Sci. 4 (4): 291–301.

[33] Bekiaris V, Withers D, Glanville SH, McConnell FM, Parnell SM, Kim M, Gaspal FMC, Jenkinson E, Sweet C, Anderson G and Lane PJL (2007) Role of CD30 in B/T Segregation in the Spleen. J Immunol. 179: 7535-7543.

[34] Horie R and Watanabe T (1998) CD30- Expression and function in health and disease. Semin. Immunol. 10: 457-47.