UJMR, Volume 6 Number 2, December, 2021, pp 30 - 36 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.2162.005
Received: 22nd August, 2021 **Accepted:** 14th October, 2021

Immunocytotoxic Effect of Aqueous Leaf Extract of *Cassia occidentalis* on Human Peripheral Blood Mononuclear Cells and Neutrophils

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Abstract

The study evaluates the potential immunocytotoxic effect of aqueous leaf extract of *Cassia occidentalis* on human peripheral blood mononuclear cells (PBMCs) and Neutrophils. Different concentrations (25 µg/ml, 50 µg/ml, 100 µg/ml) of the extract were prepared. Six millilitres (6ml) of peripheral blood from consented healthy volunteers was collected and PBMCs and neutrophils were isolated on Histopaque media. The viability of PBMCs and neutrophils was determined using trypan blue dye exclusion methods. One-way analysis of variance was used in analysing the results. Total viable cell count (TVCC) for PBMCs and neutrophils yielded 298.9 x 10^4 cells/ml and 327.9 x 10^4 cells/ml respectively. The TVCC of the highest concentration (100 µg/ml) of the extract used for the treatment of PBMCs was 192.7 x104 cells/ml and that of neutrophil was 50.47 x10^4 cells/ml equivalent to 82.51% and 61.71% mean viability percentages respectively. The PBMCs and neutrophils treated with 25 µg /ml of the extract have the highest mean percentage viability scores of 94.88% and 74.61% respectively. There was a significant difference in the mean percentage viability when control PBMCs was compared with those treated with 100 µg/ml ($p<0.0001$) and also when control neutrophils was compared with those treated with 25 µg /ml ($p=0.02$), 50 µg /ml and 100 µg /ml ($p<0.0001$) respectively. The cell viability tends to decrease in a dose-dependent manner. The aqueous leaf extract of *C. occidentalis* has a potent cytotoxic effect on both cells, especially at a higher dose. The study recommends an in-depth study to improve the credence of the present study findings.

Keywords: *Cassia occidentalis* extract, Cytotoxic activity, Human Neutrophils and PBMCs.

INTRODUCTION

Humans perpetually use medicinal plants for the treatment of disease conditions as well as other pharmacological applications on animals (Ghani, 1998). The drugs sourced from plant materials are becoming popular globally in health care settings (Bashir *et al.*, 2015). The phytoconstituents differs among the different parts of the plant materials wereuse based on purpose (Rahman *et al.*, 2013). Some of the reported chemical constituents are tannins, alkaloids, volatile oils, minerals, vitamins, Besides, every part of the *C. occidentalis* plant was used for different purposes (Sini *et al.*, 2010) and several studies indicate its application in different disease conditions. For glycosides, among others (Srivastava, 2003). However, natural products have some limitations based on their potency, safety and dose due to the paucity of reliable data (Jowell, 1999). Therefore, safety is one of the critical factors to consider before using plant materials to avoid potential adverse reactions (Shah *et al.*, 2013).

Cassia occidentalis was recognized as one of the widely accepted medicinal plants in traditional medicine, as it yields the desired effect (Yakubu *et al.*, 2007). instance, it is used as a poison antidote, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases (Vijayalakshmi *et al.*, 2013). Other

uses include anti-microbial agent (Sadiq *et al.*, 2012), antioxidant (Daniyan *et al.*, 2011), immunosuppressive and larvicidal (Abirami *et al.*, 2011) or in wound healing (Garba *et al.*, 2015), sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases, throat infection (Arya *et al.*, 2010) to mention but a few. Local communities are using the fresh leaves of the plant without prior knowledge of its effect on the body, thus necessitate an effort to assess its safety (Berinyuy *et al.*, 2015).

Presumably, the *C. occidentalis* may affect the immune system, based on its ability to triggers anti-inflammatory response with consequent production of anti-inflammatory cytokines (Koffuor *et al.*, 2016). The immune system protects the body against invading microorganisms (Bomford, 2010). It comprises several cells and cells secretion that identify and neutralize an unlimited number of threats (Naga *et al.*, 2014). Peripheral blood mononuclear cells (PBMCs) are a group of blood cells that possess a round nucleus and function to protect the body against infections. These major cells of the immune system consist of lymphocytes, monocytes, or macrophages. Neutrophils are one of the most popular groups of cells out of many that scout to protect against microbes; they are indispensable in the immune system (Kruger *et al.*, 2015).

A report shows that the *C. occidentalis* contain phytotoxins (Rekha *et al.*, 2016). Accordingly, the plants may be toxic to the immune cells, as exposure of immune cells to the toxic compounds may lead to a decrease in cell viability and cell death (Sudeep *et al.*, 2017). Indeed, a study has indicated, the use of plant extracts in high dose could lead to toxic injury to the kidneys, liver, intestine and immune cells, which interfere with normal biological functions (Mainasara *et al.*, 2016). Most of the previous studies concentrate on medicinal uses, application or properties of the plants in different research models (Kangiwa*et al.*, 2017; Tochukwu *et al.*, 2018).

Of note, people consume the extract of *C. occidentalis* for treatment of many bacterial and fungal infections; but are ignorant of the concentration they ingested. The information on human PBMCs and neutrophils viability after exposure to aqueous leaves extract of *C. occidentalis* is scarce. Consequently, this study focused on the potential immunocytotoxic effect of aqueous leaves extract of *C. occidentalis* on human PBMCs and neutrophils. This will serve as another concerted effort to provide much-needed information on this important plant.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *C. occidentalis* were collected locally. The plant taxonomic identification and assigning of specimen voucher numbers were carried out at the Department of Botany, Usmanu Danfodiyo University Sokoto, Nigeria. The voucher number assigned was UDUH/ANS/0110.

Preparation of Plants Materials

Fresh leaves of *C. occidentalis*werewashed thoroughly, shade-dried, coarsely powdered using mortar and pestle and then sieved. Subsequently, 1300 g of the dried powder was dissolved in 7 L of distilled water. We left thepreparation to soak for 24 hours in a water bath set at 40℃. Then we filtered the preparation using Whatman No. 1 filter paper. The resultant filtrate was concentrated to dryness at 40℃ under reduced pressure (Gadanya and Muhammad, 2018). A weighing quantity of 0.1 g of the dried extract was dissolved in 1 ml of distilled water, and this served as the stock.

Blood Sample Collection and Processing

Six millilitres (6 ml) of whole blood was collected from consented healthy volunteer using the Monovette vacutainer system and sample collected was transferred into a labelled lithium heparin tube, and mixed properly—the blood sample was used for isolation of human PBMCs and neutrophils.

Isolation of PBMCs and Neutrophils

The procedure was carried out according to the manufacturers' instructions. Briefly, 3 ml of Histopaque-1119 (Sigma-Aldrich® Co. UK) was added to a 15 ml conical centrifuge tube. Subsequently, 3 ml of Histopaque-1077 (Sigma-Aldrich® Co. UK) was carefully layered onto the Histopaque-1119 and brought to room temperature. Carefully 6 ml of whole blood was layered onto the upper gradient of the two different density Histopaques and centrifuged at 700 × g for 30 minutes at room temperature. PBMCs were obtained from the first layer (onto Histopaque 1077), and neutrophils were isolated from the second buffy coat layer (onto Histopaque 1119). The cells were then washed twice with RPMI 1640 (Sigma-Aldrich® Co. UK) at 200 × g for 10 minutes. The cells were re-suspended in 2 ml of RPMI 1640 media each and immediately used.

PBMCs and NeutrophilsCount (Trypan blue assay)

The cells were counted using a haemocytometer, 10 µl of 0.4% Trypan Blue (Lobal Chemie Mumbai, India) solution (w/v)

was dispensed into a 2 ml Eppendorf tube then 10 µl of each of the PBMCs and the neutrophil suspension was added into different tubes (dilution factor = 2) and mixed thoroughly, the mixture was allowed to stand for 5 minutes. With the cover slip in place, the pipette was used to transfer 10 µl of the Trypan blue-cells suspension mixture to both chambers of the haemocytometer. Both the viable and non-viable cells were counted using a microscope. Non-viable cells are stained blue, whereas viable ones remain colourless. The percentage of viable cells was calculated.

Treatment of PBMCs and Neutrophils with Plant Extract

Ten sets of 2 ml falcon tubes, five for each PBMCs and Neutrophils, were placed in a tube rack. And into each tube 50 µl of PBMCs (298.9 x 10^4 cells/ml) or neutrophils (327.9 x10^4 cells/ml) suspension was dispensed then 50 µl of each of the following concentration 25 µg/ml, 50 µg/ml and 100 µg/ml of the *C. occidentalis* plant extract were added into the tubes respectively. The tubes labelled appropriately were incubated at 37^{o}C for 30 minutes in an incubator. A tube containing the PBMCs or neutrophils suspended in RPMI 1640 media without plant extract was considered as the control. All the treatments were carried out in duplicate (Sudeep *et al.*, 2017). Cell count was carried out using Trypan blue assay after the incubation as described above.

Statistical Analysis

The data obtained were entered into SPSS version 21 (IBM, USA) for analysis. Continuous variables are expressed as mean and standard deviation (SD) or mean percentage. One-way between-groups analysis of variance (ANOVA) with Post-hoc Test (Bonferroni) was carried out to compare the different concentrations of the extract. The p-value of ≤ 0.05 was used to determine the level of statistical significance.

RESULTS

PBMCs and Neutrophils Count

The total viable cell count (TVCC) of PBMCs immediately after isolation was 298.9 x10^4 cells/ml (SD=2.91). The total viable cell count of neutrophils immediately after isolation was 327.9 x10^4 cells/ml (SD=1.07). The mean percentage viability scores of PBMCs and neutrophils before treatment were 96.00% and 96.56% respectively. As depicted from Table 1, the TVCC of the control PBMCs was 273.1 x10^4 Post-hoc comparisons using Bonferroni tests showed that there was ~~a~~ significant difference in mean percentage viability score when cells/ml (SD=4.32). The TVCC of the lowest concentration (25 µg/ml) of the*C occidentalis* extract used for the treatment of PBMCs was 204.8x10^4 cells/ml (SD=5.02) that of the highest concentration (100 µg/ml) was 192.7 x10^4 cells/ml (SD=6.33). From Table 2, the TVCC of control neutrophils was 257.7 x10^4 cells/ml (SD=9.14). The TVCC of the lowest concentration (25 µg/ml) of the *C. occidentalis* extract used for the treatment of Neutrophils was 143.9x10^4 cells/ml (SD=6.43) that of the highest concentration (100µg/ml) was 50.47 x10^4 cells/ml (SD=1.57).

Effect of the *C. occidentalis* Extracts on PBMCs.

As shown in Table 1, the mean percentage viability scores of control PBMCs was 95.16%. Those PBMCs treated with 25 µg /ml of the extract have the highest mean percentage viability scores of 94.88%, while the least TVCC was among the PBMCs treated with 100 µg /ml of the *C. occidentalis* extract (82.51%). Analysis of variance (ANOVA) revealed that there was ~~a~~ significant difference in human PBMCs' mean percentage viability scores across the different concentrations of the *C. occidentalis* extract (25 µg/ml, 50 µg/ml, 100 µg/ml) as well as control (p<0.0001). Post-hoc comparisons using the Bonferroni tests indicated that there was no significant difference in mean percentage viability score between control PBMCs and those treated with 25 µg /ml and 50 µg /ml of the *C. occidentalis* extract (p>0.05). However, there was ~~a~~ significant difference in the mean percentage viability score when control PBMCs were compared with those treated with 100 µg /ml (p<0.0001).

Effect of the *C. occidentalis* Extracts on Neutrophils.

However, from Table 2, the mean percentage viability scores of control neutrophils were95.31%. Those neutrophils treated with 25 µg /ml of the *C. occidentalis* extract have the highest mean percentage viability scores of 74.61%, while the least was among the neutrophils treated with 100 µg/ml of the extract 61.71%. Analysis of variance (ANOVA) revealed that there was ~~a~~ significant difference in human neutrophils mean percentage viability scores across the different concentrations of the extract (25 µg/ml, 50 µg/ml, 100 µg/ml) as well as control (p<0.0001).

control neutrophils were compared with those treated with 25 µg /ml (p= 0 .02), 50 µg /ml and 100 µg /ml (p<0.0001) respectively.

Table 1: Effect of different concentrations of the *C. occidentalis* extract on PBMCs viability

C. occidentalis Extract Concentration (µg/ml)	PBMCs TVCC x10^4 cells/ml Mean (SD)	PBMCs Mean viability (%)	*p*-value
Control	273.1(4.32)	95.16	<0.0001
25	204.8 (5.02)	94.88	
50	185.4 (3.97)	93.47	
100	192.7 (6.33)	82.51 ^{a***}	

TVCC: Total viable cell counts, a =Control vs. 100 µg/ml, ***p<0.0001

Table 2: Effect of different concentrations of the *C. occidentalis* extract on neutrophils viability

C. occidentalis Extract Concentration (µg/ml)	Neutrophils TVCC x10^4 cells/ml Mean (SD)	Neutrophils Mean viability (%)	*p*-value
Control	257.7 (9.14)	95.31	<0.0001
25	143.9 (6.43)	74.61^{a**}	
50	72.33 (3.53)	72.31 ^{b***}	
100	50.47 (1.57)	61.71 ^{c***}	

TVCC: Total viable cell counts, a =Control vs. 25µg/ml, b=Control vs. 50µg/ml,c= Control vs.100 µg/ml, **p=0.02, *** p<0.0001,

DISCUSSION

Assessment of the cytotoxic potential of compounds in medicinal plants intended for therapeutic use is mandatory to establish drug safety (Teixeira *et al.*, 2003). Cytotoxic compounds kill living cells through insidious genetically organized mode termed apoptosis, or through haphazard rapid disintegrative modus operandi: necrosis (Çelik, 2018). Researchers employed different methodologies for the assessment of cytotoxicity among living cells (Niles *et al.*, 2007). Medicinal plants with cytotoxic potential stand a chance in cancer therapy (Anantachoke *et al.*, 2020).

This study shows that the TVCC as well as mean percentage cell viability, tends to decreased with an increased in concentrations of *C. occidentalis* plant extract for both PBMCs and neutrophils. This suggests *C. occidentalis* possess the dose-dependent cytotoxic effect on the PBMCs and neutrophils. This study demonstrated that the mean percentage viability of PBMCs and neutrophils before treatment and among control cells were within the reported range as the normal cell viability was 90–95% as highlighted by earlier researches (Chahra *et al.*, 2016; Kalgo *et al.*, 2019; Kalgo *et al.*, 2020).

While comparing the mean percentage viability scores between groups, we observed no significant difference between low dose extract (25 µg/ml and 50 µg/ml) and control for PBMCs. On the other hand, significant differences exist at the highest extract dose concentration (100 µg/ml) in both cell groups. The indifferences may be attributed to the low dose-concentration effect of the *C. occidentalis* plant extract on the cells as such do not exert significant cytotoxicity. *In-vitro* toxicity profile of the *C. occidentalis* extract might be safe for use up to a defined effective concentration (Lombardo *et al.*, 2015).

Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of medicinal plants are potentially toxic (Akintonwa *et al.*, 2009). Indeed, drugs of plant origin are not free from toxic effects (Edziri *et al.*, 2011). The cytotoxicity witnessed on the PBMCs and neutrophils may be because of some chemical constituents that *C. occidentalis* leaves contain which exerts cytotoxic effects at specific concentrations. The presence of anthraquinones, emodin, glycosides, toxalbumin, and other alkaloids contained in *C. occidentalis* can explain the encountered toxicity (Al-Snafi, 2014). Cytotoxic compounds kill live cells (Sudeep *et al.*, 2017).

Our result findings are in line with Sudeep *et al.* (2017) which reported cytotoxicity on human lymphocytes after treatment with three different plant extract *in-vitro*. Kalgo *et al.* (2020) reported a cytotoxic effect on human PBMCs after treatment with a high concentration of aqueous stem back extract of *Vitellaria paradoxa*. Boudoukha *et al.* (2016) reported dose-dependent-elastase inhibition, degranulation, phagocytosis, and chemotaxis of

neutrophils when exposed to *Santolina chamae cyparissus* extract. Similarly, attenuation of neutrophil recruitment and lowered myeloperoxidase activity in a murine study were observed when exposed to Phytol (Silva *et al.*, 2014) or *Passiflorasub peltata* plant extracts (Shanmugam *et al.*, 2020).

Neutrophils viability decrease with increasingconcentration of stem back extracts of *Vitellaria paradoxa* (Kalgo *et al.*, 2019). However, Patil *et al.* (2010) revealed the immunostimulatory effect of neutrophils upon exposure to *Bauhinia variegata* Linn bark extract. An *in-vitro* study showed that *C. occidentalis* act as an immunosuppressant on immune cells (PBMCs and neutrophils), and the plant extract treatment itself produced toxicity (Vijayabhaskar *et al.*, 2013). Indeed, the use of other cells viability assays would improve the credence of our findings.

CONCLUSION

The study revealed ~~a~~ significant decrease in PBMCs and neutrophils viability when exposed to a higher concentration of *Cassia occidentalis* plant extract but recorded an insignificant decrease in cellular viability of PBMCs at a lower concentration. This suggests potential immunocytotoxicity with more effect at higher concentration. However, there is a need for in-depth study on the effect of *C. occidentalis* on human PBMCs and neutrophils. Notwithstanding, cytotoxicity studies on organ systems starting at a murine level can give more insight.

Conflict of Interest

Authors declare no conflict of interest

Funding

This research did not receive any funding.

REFERENCES

Abirami, D. and Murugan, K. (2011). HPTLC quantification of flavonoids, larvicidal and smoke repellent activities of Cassia occidentalis L.(Caesalpiniaceae) against malarial vectore Anopheles stephensi Lis (Diptera: Culicidae). *Journal of Phytology,* 3(2), 60-71.

Akintonwa, A., Awodele, O., Afolayan, G., Coker, H.a.B. (2009). Mutagenic screening of some commonly used medicinal plants in Nigeria. *Journal of Ethnopharmacology,* 125(3), 461-470.

Al-Snafi, A.E. (2014). The pharmacological activities of Alpinia galangal-A review. *International Journal for Pharmaceutical Research Scholars,* 3(1-1), 607-614.

Anantachoke, N., Lovacharaporn, D., Reutrakul, V., Michel, S., Gaslonde, T., Piyachaturawat, P., Suksen, K., Prabpai, S., Nuntasaen, N. (2020). Cytotoxic compounds from the leaves and stems of the endemic Thai plant Mitrephora sirikitiae. *Pharmaceutical biology,* 58(1), 490-497.

Arya, V., Yadav, S., Kumar, S., Yadav, J. (2010). Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. *Life Sciences and Medicine Research,* 9(1), e12.

Bashir, L., Shittu, O., Busari, M., Sani, S., Aisha, M. (2015). Safety Evaluation of Giant African Land Snails (Archachatina maginata) Haemolymph on Hematological and Biochemical Parameters of Albino Rats. *Journal of Advances in Medical and Pharmaceutical Sciences,* 3(3), 122-130.

Berinyuy, E., Lawal, B., Olalekan, L., Olalekan, I., Yusuf, A., Sakpe, S., Ossai, P. (2015). Hematological Status and Organs/Body-weight Parameters in Wister Rats during Chronic Administration of Cassia occidentalis. *International Blood Research and Reviews,* 4(3), 1-7.

Bomford, R. (2010). Ethnomedicine: A Source of Complementary Therapeutics. *Research Signpostt,* 159, 227-244.

Boudoukha, C., Bouriche, H., Ortega, E., Senator, A. (2016). Immunomodulatory effects of Santolina chamaecyparissus leaf extracts on human neutrophil functions. *Pharmaceutical biology,* 54(4), 667-673.

Çelik, T.A. (2018). Introductory Chapter, In: Çelik, T.A. (ed). Cytotoxicity. IntechOpen Limited, 5 Princes Gate Court, London, SW7 2QJ, UK.

Chahra, D., Ramdani, M., Lograda, T., Chalard, P., and Figueredo, G. (2016). Chemical composition and antimicrobial activity of Evernia prunastri and Ramalina farinacea from Algeria. Issues in Biological Sciences and Pharmaceutical Research 4(5), 35-42.

Daniyan, S., Oloruntimelehin, J., and Ifeadi, O. (2011). Antibacterial activity of *Cassia occidentalis* flower vegetable extract on selected bacteria. Asian Journal of Biomedical and Pharmaceutical Sciences 1(1).

Edziri, H., Mastouri, M., Mahjoub, A., Anthonissen, R., Mertens, B.,

Cammaerts, S., Gevaert, L., Verschaeve, L. (2011). Toxic and mutagenic properties of extracts from Tunisian traditional medicinal plants investigated by the neutral red uptake, VITOTOX and alkaline comet assays. *South African Journal of Botany,* 77(3), 703-710.

Gadanya, A. and Muhammad, S. (2018). Hypolipidemic effect of oral administration of aqueous leaf extract of Senna occidentalis in rats. *Nigerian Journal of Basic and Clinical Sciences,* 15(1), 68.

Garba, R., Saidu, A., Adeyemi, H., and Muhammad, H. (2015). Effect of methanolic extract of *Cassia occidentalis* L. root bark on body weight and selected biochemical parameters in alloxan induced diabetic rats. British Journal of Pharmacology and Toxicology 6(2), 39-49.

Ghani, A. 2003. *Medicinal plants of Bangladesh: chemical constituents and uses*, Asiatic society of Bangladesh, 138, 1-16.

Jowell, T. (!999). House of Commons official report (Hansard). *Herbal medicines*, 26, 426-427

Kalgo, M.U., Hamid, K.M., Muhammad, U.A., Balarabe, A., Yeldu, M.H., Yahaya, I.S., Kalgo, Z.M., Aliyu, B., Bala, Y.G. (2019). Effects of Aqueous Stem Bark Extract of *Vitellaria paradoxa* on Human Neutrophil Function and Viability. *International Journal of Biological and Medical Research,* 10(3), 6782-6787.

Kalgo, M.U., Hamid, K.M., Balarabe, A., Muhammad, U.A., Bala, Y.G., Raji, H.Y., Yusuf, A., Isiyaku, A., Bagudo, I.A., Usman, A. (2020). Cytotoxic Effect of Aqueous Stem Bark Extract of *Vitellaria paradoxa* on Human Peripheral Blood Mononuclear Cells. *IOSR Journal of Dental and Medical Sciences*, 19(2 Ser.4), 48-52

Kangiwa, M.A., Nnaemeka, A.M., Oluwabusuyi, O.J., Bailade, T.E., Oluchi, O.-N.D., Iyioku, U.U. (2017). Effects of *Cassia occidentalis* on Inflammatory cells of Wister Rats Infected with *Salmonella Typhi*. *Annals of Medical Laboratory Science,* 1(1), 1-14.

Koffuor, G.A., Abruquah, A.A., Audu, R., Amoah, J., Agwah, D. (2016). Patronage and perceived efficacy of herbal antityphoid preparations, and anti-*Salmonella* activity of a herbal preparation used in Ghana. *Journal of applied pharmaceutical science,* 6(3), 1-7.

Kruger, P., Saffarzadeh, M., Weber, A.N., Rieber, N., Radsak, M., Von Bernuth, H., Benarafa, C., Roos, D., Skokowa, J., Hartl, D. (2015). Neutrophils: between host defence, immune modulation, and tissue injury. *PLOS Pathogens,* 11(3), e1004651.

Lombardo, M., Kiyota, S., Kato, E.T.M., Mathor, M.B., Pinto, T.D.J.A., Kaneko, T.M. (2015). Evaluation of in vitro biological properties of Senna occidentalis (L.) Link. *Acta Scientiarum. Biological Sciences,* 37(1), 9-13.

Mainasara AS, Oduola T, Musa U, Mshelia AS, Muhammed OA, Ajayi AS. (2011). Effect of *Vitellaria paradoxa* Stem Bark Ingestion on Kidney Functions in Wistar Rats; *British Journal of Pharmacuetical Research,* 1(2), 1-8.

Naga, P., Rajeshwari, P. (2014). An overview on immunomodulators. *International Journal of Current Pharmaceutical and Clinical Research,* 4(2), 108-114.

Niles, A. L., Moravec, R. A., Eric Hesselberth, P., Scurria, M. A., Daily, W. J., and Riss, T. L. (2007). A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. Analytical Biochemistry 366(2): 197-206.

Patil, J., Jalalpure, S., Hamid, S., Ahirrao, R. (2010). In-vitro Immunomodulatory Activity of extracts of *Bauhinia vareigata* Linn bark on Human Neutrophils. Iranian Journal of Pharmacology and Therrapeutics, 9(2) 41-46.

Rahman, M.S., Moly, N.N., Hossen, M.J. (2013). Antimicrobial, Cytotoxic and Antioxidant Activity of The Exudate Of Calotropis gigantea. *International Journal of Pharmaceutical Sciences and Research,* 4(2), 745.

Rekha, U., Thomas, J., Thomas, V., Tiju, J., Prakash, P., Latha, M. (2016). Therapeutic potential of the phytochemicals in Cassia occidentalis-a review. *European Journal of Pharmaceutical and Medical Research,* 3(9), 180-188.

Sadiq, I.S., Shuaibu, M., Bello, A.B., Tureta, S.G., Isah, A., Izuagie, T., Nasiru, S., Kamaru, M.B. (2012). Phytochemistry and antimicrobial activities of Cassia occidentalis used for herbal remedies. *Journal of Chemical Engineering,* 1(1), 38-41.

Shah, A., Marwat, S., Gohar, F., Khan, A., Bhatti, K., Amin, M., Din, N., Ahmad, M., Zafar, M. (2013). Ethnobotanical study of medicinal plants of semi-tribal area of Makerwal and Gulla Khel (lying between Khyber Pakhtunkhwa and Punjab Provinces), Pakistan. *American Journal of Plant Sciences,* 4(1), 98-116.

Shanmugam, S., Thangaraj, P., Dos Santos Lima, B., Trindade, G.G.G., Narain, N., Mara De Oliveira, E.S.A., Santin, J.R., Broering, M.F., Serafini, M.R., Quintans-Júnior, L.J., Antunes De Souza Araújo, A. (2020). Protective effects of flavonoid composition rich P. subpeltata Ortega. on indomethacin induced experimental ulcerative colitis in rat models of inflammatory bowel diseases. *Journal of ethnopharmacology,* 248, 112350.

Silva, R.O., Sousa, F.B.M., Damasceno, S.R., Carvalho, N.S., Silva, V.G., Oliveira, F.R.M., Sousa, D.P., Aragão, K.S., Barbosa, A.L., Freitas, R.M. (2014). Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundamental and clinical pharmacology,* 28(4), 455-464.

Sini, K., Karpakavalli, M., Sangeetha, P. (2010). Analgesic and antipyretic activity of Cassia occidentalis Linn. *World Applied Sciences Journal,* 11(10), 1216-1219.

Srivastava, S. K., Xiao, D., Lew, K. L., Hershberger, P., Kokkinakis, D. M., Johnson, C. S., Trump, D. L., and Singh, S. V. (2003). Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits growth of PC-3 human prostate cancer xenografts in vivo. *Carcinogenesis,* 24(10), 1665–1670.

Sudeep, N., Nithya, M., Kiranmayee, P. (2017). Evaluation of in vitro cytotoxic effects of three medicinal plants on peripheral blood mononuclear cells (PBMCs). *Journal of chemical and pharmaceutical research,* 9, 18-26.

Teixeira, R.D.O., Camparoto, M.L., Mantovani, M.S., Vicentini, V.E.P. (2003). Assessment of two medicinal plants, Psidium guajava L. and Achillea millefolium L., in in vitro and in vivo assays. *Genetics and Molecular Biology,* 26(4), 551-555.

Tochukwu, N., Kenneth, A., Somadina, O., Chukwuebuka, N., Chigozie, I. (2018). Curative and Protective Efficacy of Cassio occidentalis Ethanolic Leaf Extract on Carbon Tetrachloride Induced Liver Damage of Female Albino Wistar Rats. *EC Clinical and Experimental Anatomy,* 1(2018), 48-61.

Vijayabhaskar, K., Chaitanyaprasad, K., Srisailam, K., Arunadevi, N.M., Swathi, S., Subhashini, P. (2013). Analgesic and anti-inflammatory activities of the extract of Cassia occidentalis (Linn.) animal model. *International journal of research in pharmacy and chemistry,* 3(4), 759-762.

Vijayalakshmi, S., Lingam, R., Rajeswari, D., Bhagiyalakshmi, M. (2013). Pharmacological profile of *Cassia occidentalis* L - A review. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5, 29-33.

Yadav, J.P., Arya, V., Yadav, S., Panghal, M., Kumar, S., Dhankhar, S. (2010). Cassia occidentalis L. A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia,* 81(4), 223-230

Yakubu, M., Akanji, M., Oladiji, A. (2007). Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacognosy Magazine,* 3(9), 34-38.